

Effects of different sample processing protocols on dermatophyte culture yield: A comparative study

Jaswinder Singh Gill^{1,*}, Sourav Sen², Ashwini Agarwal³

¹Associate Professor, ²Professor and HOD, ³Professor, Dept. of Microbiology, Armed Forces Medical College, Pune, Maharashtra, India

*Corresponding Author: Jaswinder Singh Gill

Email: jsjsgill02@gmail.com

Abstract

Introduction: Dermatophytes fungi infect keratinized tissues such as hair, nails and skin. Different methods for sample collection and transportation of dermatophytes for culture have been described. The standard methods for dermatophytes sample collection, transportation and lab processing, results in low positive culture dermatophyte yield. In this study, we have compared the standard method (Protocol A) with modified sample processing protocol (Protocol B), developed by our department. This modification was based on the core concept of collection, inoculation, starting incubation and transportation of dermatophyte sample, at patient's point of care.

Materials and Methods: A total of 200 clinical isolates of dermatophytes, were subjected to two different sample processing protocols. Standard method was Protocol A and laboratory developed method was labelled as Protocol B. Protocol B, included a small (7ml) transparent plastic tube with special culture media. These tubes were inoculated with sample at patient's point of care and same tube were utilized as transportation media.

Results: Samples processed as per modified protocol (Protocol B), demonstrated positive dermatophyte culture yield in 160 out of 200 samples, whereas figures were 80 out of 200 for samples processed as per standard protocol (Protocol A). Difference in positive dermatophyte culture yields, were statistically significant. The contamination rates of dermatophyte culture by saprophytic fungus and bacteria, was measured, 6% for Protocol A and 1% for Protocol B.

Conclusion: Our study demonstrated that a simple, economical modification in the procedure for dermatophyte sample processing by the laboratory, has led to a significant increase the positive dermatophyte culture yield with reduced secondary contamination rates.

Keywords: Dermatophytes, Sabouraud dextrose agar, Fungal culture, Contamination.

Introduction

Dermatophytes are the unique group of fungi that can invade the keratinized tissues. Keratinized tissue infected by dermatophytes includes skin, hair, and nails.¹ Dermatophytes exist in two phases during their life cycle, as most other fungus species do. These states are the anamorph state (imperfect or asexual phase) and the teleomorph state (perfect or sexual phase). State isolated in the laboratory is anamorph state. As teleomorphs state is difficult to detect and identify, it has yet not been described for many species of dermatophyte. Fungus species in Dermatophytes belongs to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*.²

Based on their natural habitats, Dermatophytes are broadly classified as geophilic, zoophilic and anthropophilic species. Among geophilic species, *M. gypseum*, a soil-inhabiting saprophytes can occasionally be pathogenic to humans. Animals are infected by zoophilic species and humans are accidental hosts. These group of dermatophytes has acquired the ability, from degrading keratinous debris in the soil to invading keratinized tissue of the animal host. Zoophilic species includes *T. equinum*, *T. verrucosum*, *T. mentagrophytes* and *M. canis*. Anthropophilic species like *T. rubrum*, have evolved from zoophilic fungi to infect human host.³

Dermatophyte infections can involve different areas of the body with varied clinical manifestations. These clinical disease are named using word tinea followed, a Latin term for the specific body part such as Tinea corporis (non-hairy skin), Tinea capitis (scalp), Tinea cruris (groin), Tinea pedis (foot) and Tinea barbae (bearded areas).⁴

Tinea pedis is characterized by infection of the interdigital web spaces and feet. These lesion presents as vesicles or pustules lesions. Common etiologic agents are *E. floccosum*, *T. mentagrophytes* and *T. rubrum*.⁵ Tinea capitis is dermatophyte species involving hair shaft either in ectothrix, or endothrix manner. Mainly *M. canis* and *M. audouinii* are known for ectothrix infection, whereas endothrix type involvement is by *T. tonsurans*.⁵ Tinea corporis is caused by *T. rubrum*, *T. mentagrophytes* and *M. canis*. Tinea corporis presents as annular lesions with raised borders.⁵

Tinea unguium is characterized by the nail involvement presenting as thickened, discolored nails with subungual debris. Common etiological agents are *T. rubrum*, *T. mentagrophytes* and *E. floccosum*. Superficial onychomycosis is mainly caused by *T. mentagrophytes*.⁶

Due to warm humid condition, dermatophyte infection is a common clinical manifestation. It has high prevalence worldwide as the most common fungal

pathogens infecting humans.⁷ Majority of these infection can be treated with topical application of antifungal agents, however some of these infections especially onychomycoses requires long term treatment due to poor responsive to standard treatment.⁸ Mycological examination for species level identification of dermatophytes, is required preferably in fresh cases and mandatory in unresponsive superficial mycosis cases.⁹ This is also important in view of recent reports of Azole resistance in the dermatophytes isolates.¹⁰ As per the standard procedure, superficial mycosis sample materials are transported in dry, hard black paper which is folded in a herbarium packet and transferred to the laboratory.¹¹ At the Mycology laboratory dermatophyte species level identification is performed by microscopic examination and culture characteristic.¹¹ For dermatophyte culture special media like Sabouraud dextrose agar (SDA), Sabouraud Dextrose Agar containing chloramphenicol and cycloheximide and Dermatophyte test medium may be used.¹² However, by these standard methods positive dermatophyte culture yield is low.¹³ This low culture yield is further complicated by frequent secondary contamination by saprophytic fungus or bacteria during transportation.¹⁴ In our department, we have prepared new protocol, with concept of collection, inoculation and incubation of sample at the point of care. In this study, we have compared the effects on dermatophyte culture yield based on two different protocols- Standard protocol (Protocol A) and point of care protocol (Protocol B).

Materials and Methods

In this study, 200 consecutive, non repeat samples from clinically diagnosed dermatophyte patients reporting to a tertiary care hospital were included. Each patient samples were processed as per two different protocols. These protocols were designated as Protocol A and Protocol B. Standard operating procedures of these protocols were issued, which are as follows:-

Protocol A- Standard Protocol

Collection of Sample: All clinically diagnosed lesion of dermatomycosis was cleaned with 70% alcohol before sample collection. For the skin lesions, sterile blunt scalpel was used and scales from lesion margins were collected. Infected basal portion of hairs were plucked with sterile forceps. For patients presenting with onychomycosis, nail clipping or scrapings were collected with a scalpel blade. All the above samples were placed in a dry black paper.

Transport of Sample: Sample material was transported to mycology laboratory in the dry, hard black paper, which was folded in a herbarium packet. The samples were divided into two portions: one for microscopic examination and one for culture.

Laboratory Processing

Microscopy Examination: Samples were received in black paper for direct microscopic examination.

Solution for wet mount preparation was prepared in two different strengths and kept in different clean bottles. These preparation were of 10% potassium hydroxide (KOH) was mixed equal quantity of 40% Dimethyl sulphoxide (DMSO) and 40% KOH with equal quantity 40% DMSO. Skin and hair specimen were processed, using 10% KOH mixture and for nail samples, 40% mixture was used. Before microscopic examination, 3-4 drops of the above mixture was placed on a clean glass slide. The representative clinical sample of dermatophyte were placed into the KOH+DMSO drops on the slide. A cover slip was placed carefully to avoid any air bubble formation. Potassium hydroxide (KOH) in presence of DMSO softens keratin present in tissue. After 10 minutes, each slides were examined both under low power (10X) and high power (40X) magnification for the presence of fungal elements including arthroconidia.

Dermatophyte Culture: All clinical samples were streaked on the Sabouraud Dextrose Agar (SDA) slants with cycloheximide (50 mg/l) and chloramphenicol (500 mg/l). These culture slopes were placed in incubator and temperature of 30°C was maintained. These samples were incubated for the duration of 4 weeks. Identification of dermatophyte with positive growth was done utilizing both macroscopically and microscopically features. Macroscopic features included dermatophyte colony characteristics, whereas microscopically features included study of type of dermatophytes conidia, including its size, surface characteristics and septations.

Protocol B- Point of Care Protocol

Description of the Modified Culture and Transport

Media: As per this protocol the core concept was to collect, inoculate and start incubation at the point of care, so a modified culture and transport media was prepared (Fig. 1 and 2). The modified culture and transport media included a small, soft, transparent, plastic tube of 7 ml capacity with tightly fitting screw cap. These special tubes were filled with 3ml slant of Sabouraud Dextrose Agar with chloramphenicol (50mg/l) and cycloheximide (500mg/l). As these tubes had tightly fitting screw cap loss of oxygen and water vapor was minimal. Cycloheximide was added to inhibit saprophytic fungi and chloramphenicol was added to inhibit bacteria growth.

Collection of Sample: As per protocol A

Inoculation and Transport of Sample: Sample were immediately inoculation on to the medium and screw cap placed. As dermatophytes grow at room temperature, these tube were kept upright during transportation.

Laboratory Processing: Sample received in special culture and transport media were not opened. They

were placed in incubator directly for incubation at 30° C. The special culture tube slopes were examined regularly, for any evidence of fungal growth. Cultures were incubated for 1 month before discarding them as negative. Identification of fungal growth was performed as per standard methods, which includes colony morphology; pigment production and microscopic examination.



Fig. 1: Modified dermatophyte sample collection and transportation media



Fig 2: Growth of dermatophytes in modified collection and transportation media

Results

Among 200 consecutive, non repeat clinical samples collected, 75% (150 out of 200) were from male patients and 25% (50 out of 200) were from female patients. Age distribution of patients presenting with dermatophytosis infection, in our study is shown in table 1. The distribution of clinical presentation among the patient included in our study is shown in table 2. All these 200 consecutive fungal samples processed as per Protocol A, revealed positive dermatophyte culture yield in 80 (40%) out of 200 samples. Samples processed as per Protocol B, demonstrated 80% positive dermatophyte yield. For comparisons, the positive dermatophyte culture yield by two different protocols, were analyzed by statistically method- McNemar exact. Null hypothesis stating that no significant difference between two protocol was rejected ($P < 0.05$). Contaminations of cultures slope by saprophytic fungus and bacteria was noted in 12 tubes for samples processed by the protocol A. So the contamination rate for protocol A was 6%, whereas it was 1% for protocol B.

Table 1: Age distribution of clinical dermatophytes patient included in the study

Age group	Number of positives	Percentage
0–10 years	2	1%
11–20 years	20	10%
21–30 years	76	38%
31–40 years	64	32%
41–50 years	34	17%
51–60 years	3	1.5%
Above 60 years	1	0.5%

Table 2: Clinical manifestation of dermatophyte infection

Dermatophyte infection	Number of cases	Percentage
<i>T. corporis</i>	134	67%
<i>T. pedis</i>	30	15%
<i>T. cruris</i>	26	13%
<i>T. capitis</i>	6	3%
<i>T. unguium</i>	4	2%

Discussion

Due to humid environment, dermatophytosis is most common superficial fungal infection in tropical and subtropical countries, like India.¹⁵ Diagnostic Laboratory with facility for rapid and accurate identification of the dermatophytes are essential for definitive treatment and planning prevention measures. In our study, 200 consecutive, non-repeat clinically samples were collected and processed by two different protocols- protocol A and B. These samples included clinical cases of *T. corporis* (67%), *T. pedis* (15%), *T. cruris* (13%), *T. capitis* (3%) and *Tinea unguium* (2%). This finding is in concordance with other studies conducted in India.¹⁶ In our study, 21-30 years (76/200) age group patient were the most common, followed by patients from age group of 31-40 years (64/200). Similar findings have been reported by other Indian authors.¹⁷

Diagnostic laboratories receives clinical samples from patients with dermatophytosis, which generally includes skin, nails, and hair. These samples requires careful collection and transportations. Typically, a black paper placed in envelope is used for transportation. Many authors have described, different techniques for the sample collection such as scraping, cotton swabs, cellophane or vinyl strip for skin,¹⁸ hair clipping or brush technique for hair¹⁹ and clipping and microdrill for nail.^{20,21} However, the patient samples are contaminated with saprophytic fungi and bacteria²² at the time of collection and gets secondary contamination from environment during transportation and handling of sample at the diagnostic laboratory.²³ This high risk of secondary contamination, from the environment, during transportation and sample processing warrants use of special culture medium in the laboratory. Various

studies have shown high contamination rates and low fungal culture yield, despite using special mediums like Sabouraud dextrose agar supplemented with chloramphenicol and cycloheximide.^{14,24} Similar findings of high contamination rate (6%) and low dermatophyte culture yield (40%) was observed in our study, for the samples processed as per standard procedures (Protocol A).

In this study, new protocol (Protocol B) was designed with core concept of sample collection, inoculation, transportation and starting the incubation in the special fungal culture medium for dermatophytes, right at the point of care. This modification of the procedure, resulted in statistically significant increase in positive dermatophyte culture yield (80%). Our study also demonstrated the reduction in the secondary contamination rate of the dermatophyte fungal culture to 1% compared to 6% for the standard procedure. This observable reduction in contamination rate may be multifactorial, among which important ones are reduction in the primary and secondary contamination of the samples by saprophytic fungus and bacteria. Primary contamination was minimized, as sample were collected after cleaning infected area with 70% alcohol. Secondary contamination was reduced as sample were placed inside the screw cap tubes with culture media at the point of care. Further, secondary contamination was avoided due to screw capped dermatophyte culture tubes and by avoiding sample handling at the laboratory, until the growth of pathogenic dermatophyte.

In resource constrain laboratory, this modification may be beneficial as media dispensed per tube was merely 4 ml against 8ml to 15ml medium required for the standard procedure.

Our modification has resulted in higher rate of isolation of dermatophytes in our laboratory, which has translated into better patient management, improved surveillance, effective planning of preventive measures and further research, as dermatophyte antifungal susceptibility pattern of local population.

Conclusion

Our study shows that the simple economical modification in the procedure for dermatophyte sample processing standards, has significantly increased the positive culture yield with reduced secondary contamination rates. This modification of dermatophyte sample processing protocol has potential for positive ramification on patients health, over a long duration, as it has basis of scientific and evidence based management of patients presenting with dermatophyte infection.

Conflict of Interest: None.

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