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ISOLATION, IDENTIFICATION AND COMPARATATIVE STUDY OF SDA AND DTM FOR DERMATOPHYTES FROM CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL, KANCHIPURAM

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ABSTRACT

Back ground: The study on dermatophytes based on their morphology by 10-20 %KOH mount, cultural characteristics on various media, Slide culture are useful for their prevention as well as cure as these infections are most common and associate with significant morbidity and disfigurement. Though dermatophytic infections are treated based on clinical manifestations alone, because of the delay in their isolation by using fungal culture media,(7-14 days) t,he clinical manifestation of the infection alone is not useful to identify the causative organism. Thus a study is conducted on isolation, identification and comparison between two fungal isolation media. **Materials & methods:** 10 -20%KOH, Dermatophyte medium, SDA are used for the study. **Results:** Among 220 clinically diagnosed cases of superficial mycotic infections 132 cases were dermatophytic infections 88 cases were due to other .66.6% were males and 44.4% were females. commonest infection being Tinea corporis(54.28%) and the commonest species isolated were Trichophyton rubrum.(51.45%).The cases were common during November and December months. In present study cultivation by using DTM was sensitive (76.08%) than SDA medium.(71.73%) and 10 –20% KOH mount (54.34%). **Conclusions:** By using dermatophyte medium the identification of various dermatophytes can be made accurately and specifically. More over because of incorporating cycloheximide and chloramphenicol the contamination is reduced.

KEYWORDS: dermatophytes, commonest infection, cycloheximide and chloramphenicol.

INTRODUCTION

Dermatophytic infections are caused by three genera of fungi Trichophyton, Microsporum, Epidermophyton[1] that infect superficial keratin layers[2] such as skin,hairs,nails.Severe form of infections are seen in immune compromised individual and are associated with trivial complications.[3,4] The commonest species being T.rubrum, T.mentagrophyte, M.audouinii, M.canis, Epidermophyton floccosum.[5]

The fungal infection is worldwide in distribution and one among the common medical condition.[1,2] in general as well as in our hospital. Even though there is rapid evolution in molecular diagnostic techniques, microscopy and cultivation for species identification are still conventional confirmatory diagnostic methods. They have characteristic colony morphology cottony(M.canis, T.mentagrophytes), velvety (M.audouinii), powdery (E.floccosum) with some colonies showing typical pigment red (T.rubrum) coloured, violet coloured (T.violaceum). They are identified by macroconidia (Epidermophyton floccosum, Microsporum) or microconidia (Trichophyton rubrum(bird on fence like)Trichophyton mentagrophytes(engrappe). Some dermatophyte such as T.mentagrophytes have peculiar properties hair perforation, urease test positivity by which they can be identified.[6,7]

The infections cuased by dermatophytes are known as ring worm infections that include T.corporis(body), T.pedis(foot),T.cruris(buttocks),T.unguum(nails), T. barbae(beard), T. capitis(head), T. concetricum (bod, T.incognito(inadvertent therapy with steroids). They often confused with other superficial fungal infections such as P.versicolar, candida, Piedra etc.,. Thus for their specific management definitive identification is essential.[8]

A study was carried out to compare various methods of identifying dermatophytes including10-20%KOH mount, slide culture, dematophyte medium, SDA .[6.7]

MATERIALS AND METHODS

10 -20% KOH mount

The material (skin scrapings,infected nails,infected hair with intact root)is placed in 10 – 20% KOH preparation

for few minutes .For nail sample 20%KOH is used and the sample in 20%KOH is warmed under Bunsen flame before being examined. In present study only one case of T.manuum was sent for dermatophyte culture.

Dermatophyte medium

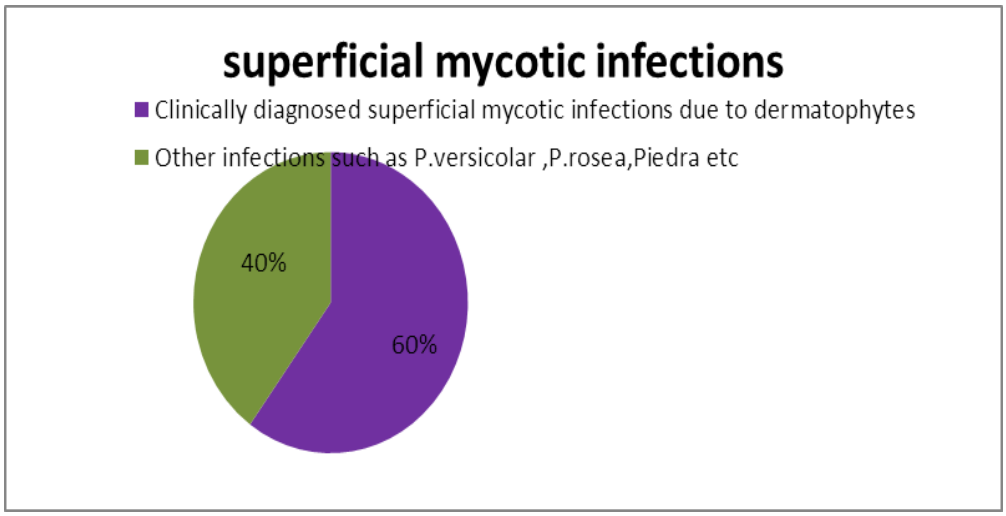
Dermatophyte medium composition was of papaic digest of soybean meal, dextrose, cyclohexamide, phenol red, chloramphenicol, Agar at final Ph 5.6 +/- 0.2 and the media is poured on to a petridish /test tube. The inoculated media is incubated at 25 degree C. Observed for growth after 24 hours every day upto 7 days. Interpretation of results: Red colour around the colony suggests positive for dermatophyte and growth without colour change indicates non-dermatophyte.

SDA medium Sabourauds dextrose agar
Peptone, water, Dextrose with PH 5.6

RESULTS

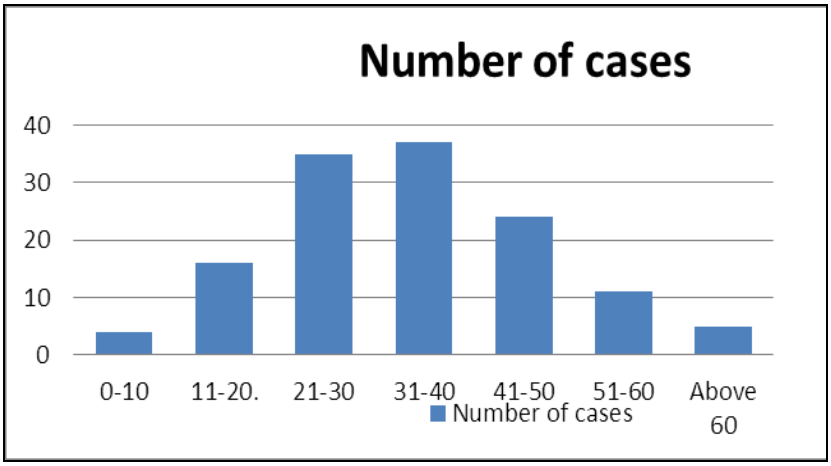
In our present study all clinically diagnosed cases of superficial mycotic infections starting from October 2014 to August 2015 attending a dermatology OP in a tertiary care hospital were included. Among 220 clinically diagnosed cases of superficial mycotic infections 132 cases were clinically diagnosed as dermatophytic infections.Rest of them (88cases) were due to various other superficial mycotic infections such as P.versicolar, P.rosea, Piedra etc., that are included under exclusion criteria in our study.

	Number of cases	Percentage
Clinically diagnosed superficial mycotic infections due to dermatophytes	132	60%
Other infections such as P.versicolar, P.rosea, Piedra etc	88	40%
Total number of cases	220	



Age distribution of various clinically diagnosed dermatophytic infections

s.no	Age	Number of cases
1.	**0-10**	**4(3.03%)**
2.	**11-20**	**16(12.12%)**
3.	**21-30**	**35(26.51%)**
4.	**31 – 40**	**37(28.03%)**
5.	**41 – 50**	**24(18.18%)**
6.	**51 – 60**	**11(8.3%)**
7.	**Above 60**	**5 (3.7%)**
Total	**132**	



Out of 220 cases 132 cases were due to different dermatophytic infections and 88 cases were due to pytiriasis versicolar. In present study only dermatophytic infections are included. Among 132 cases 88 (66.6%)cases were male patients and 44(33.3%) cases were female patients.

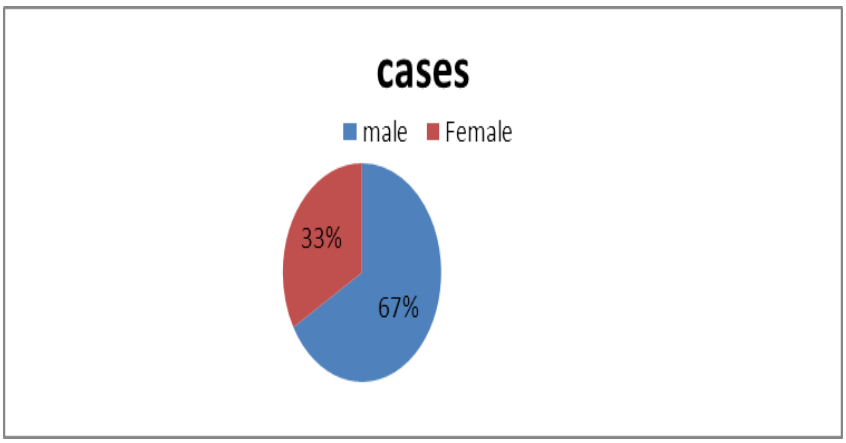
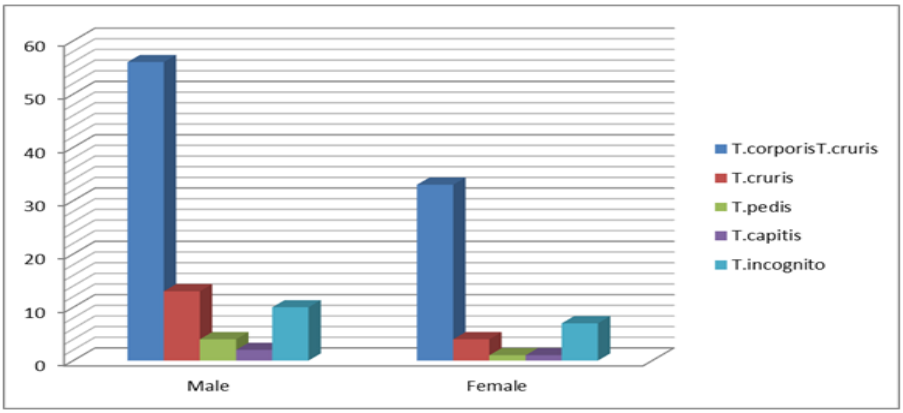


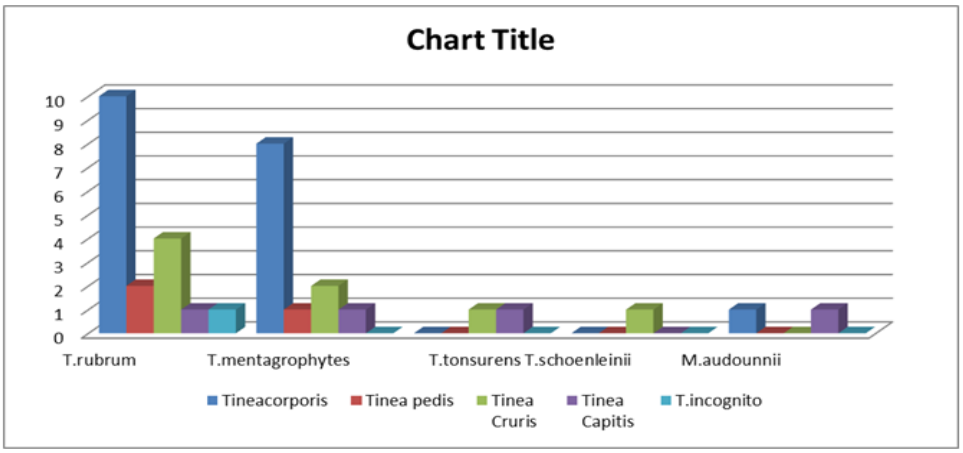
Table-1
SEX DISTRIBUTION OF DIFFERENT CLINICAL TYPES OF DERMATOPHYTES
Tinea corporis infections were found to be the most common

Clinical types	Male	Female	Total
T.corporis	**56**	**33**	**89**
T.cruris	**13**	**4**	**17**
T.pedis	**4**	**1**	**5**
T.capitis	**2**	**1**	**3**
T.incognito	**10**	**7**	**17**
Total			**132**



Out of 46 samples collected upon request from dermatologist for 10% KOH mount and fungal culture 35 samples(76%) have given rise to different fungal isolates that include Trichophyton rubrum, T.mentagrophytes T. schoenleinii, M. audounnii, M.gypseum. 11 samples were culture negative. (24%). Among culture negative cases many were from cases of T.incognito.out of 46 samples 25 samples (54.34%) showed the presence of fungal elements. 21 samples(45.66%) were negative for 10%KOH mount.

Dermatophytes isolated	**Tinea corporis**	**Tinea pedis**	**Tinea Cruris**	**Tinea Capitis**	**T.incognito**	**Total**
T.rubrum	**10**	**2**	**4**	**1**	**1**	**18(51.45%)**
T.mentagrophytes	**8**	**1**	**2**	**1**	**--**	**12(34.28%)**
T.tonsurens	**-**	**0**	**1**	**1**	**-**	**2(5.7%)**
T.schoenleinii	**-**	**0**	**1**		**-**	**1(2.8%)**
M.audounnii	**1**	**0**	**-**	**1**	**-**	**2(5.7%)**
total	**19(54.28%)**	**3(8.5%)**	**8(22.8%)**	**4(11.4%)**	**1(2.8%)**	**35**



Comparison of culture positivity and KOH positivity

	KOH positive	**KOH negative**	**Culture positive**	**Culture negative**
Total number of samples processed46	25	21	35	11
Culture positivity in KOH positive cases	25	-	25	nil
Culture positivity in KOH negative cases	-	21	10	11

Total dermatophytes isolated - 35

The two sets of SDA and DTM were inoculated with properly collected sample from growing edge of the lesion and incubated at 25°C and 37°C.The growth in DTM had resulted in colour change to red colour because of the presence of phenol red indicator and due to production of alkaline biproducts. The growth was further studied for morphology of colony, rate of growth, typical microscopic morphology, (macroconidia. microconidia) Further morphology is confirmed by slide culture. speciation is done based on microscopic morphology and hair perforation test, urease test.

Christesen urease medium: with 1% glucose were inoculated with growth obtained from primary isolation media for 7-14 days incubated at 25°C.

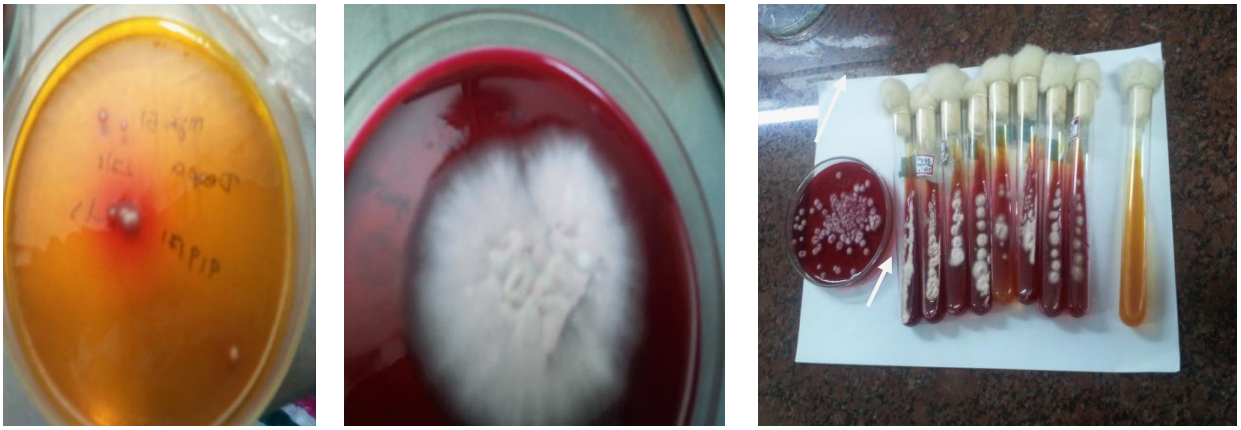
Hair perforation test

The hair were inoculated sterilised and placed in distilled water with sterile yeast nitrogen base. The colonies of dermatophytes are inoculated by touching the hair and incubated at room temparature for 14 – 20 days T.mentagrophyte were positive for urease test and hair perforation test.

COMPARISON OFNUMBER OF CULTURE POSITIVES ON DTM AND SDA

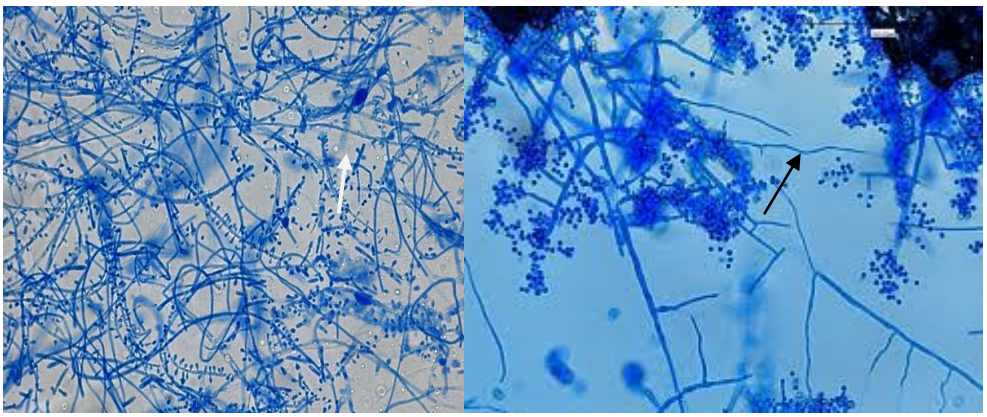
	Positive cases	Percentage
Total number of cultures	46	-
Total number of dermatophytes	35	76.08%
Cutures positive by DTS	35	76.08%
cultures positive by SDA	33	71.73%

Out of 46 culture for isolating dermatophytes 35 were culture positive and were isolated on DTM (76.08%)where as 11 were culture negative. whereas on SDA 71.73% i.e.,33 cases were isolated .



Different dermatophytes isolated on special dermatophyte media showing colour change

T.rubrum on DTM Growth of M.canis and T.mentagrophytes on SDA



T.rubrum showing microconidia T.mentagrophytes with microconidia

DISCUSSION

Most of the dermatophytic infections are usually diagnosed and treated based on clinical presentations alone, as it takes long time to obtain culture report Microscopic examination of the sample often needs expertise to identify fungal elements with dermatophyte

morphology. However it offers painless and rapid results. As many of the non-infective conditions often present with similar clinical presentation, culture identification of deramtopthytic infections are usually recommended for those cases that require prolonged treatment with antifungal agents.[4] In our present study also out of 132 cases clinically diagnosed as dermatophytic infections only 46 cases were requested for culture confirmation for dermatophytes and for 10% KOH mount..Among 132 clinically diagnosed cases male to female ratio was 1:2 i.e., 88 :44.This finding is correlating with many studies.[9,10] The age distribution is also correlating to several studies.i.e., more common among 30-40 years age group.[11,12] Seasonal distribution more common during October to December months.out of 46 cases 35 cases were culture positive i.e., 76% and 24% were culture negative on special dermatophyte media whereas 25 cases (54.34%)were positive for 10% KOH mount and 21 cases were negative .(45.66%). This is correlating with other studies.[13,14,15] 59.20%, 53.3%, 49%, however some of the studies show that 10%KOH preparation is more sensitive.[16,17,18] The commonest infections were Tinea corporis(54.28%) and the commonest causative organism was Trichophyton rubrum(51.45%). This is correlating with many other studies in India.[19] In present study the 10%KOH mount showed 54.34% positive i.e.,25 positives out of 46 samples where as culture positive were 35 out of 46 clinical cases(76%).All 10%KOH positive cases were positive even for culture. This can be attributable to personel collection of sufficient samples from active growing edge of the lesion& their proper transportation .According to some studies 10%KOH is more sensitive than fungal culture.[20] however in our study fungal culture was more sentive(76%) this is correlating other studies where culture positive was 79.1%(3) 62.8%.[16]

By using dermatophyte media with chloramphenicol and cycloheximide the contamination is reduced to minimum. The growth is soon identified by colour change in the medium because of production of alkaline biproducts by growth of dermatophyte and non-dermatophyte produces no colour change.SDA medium there is greater chances for contamination and by the time the appears the medium is subjected to dehydration.[21]

CONCLUSION

The dermatophytic infections are common in males with age group 30-40years and are common among superficial infections.For laboratory identification& diagnosis even though microscopy is Gold standard, isolation by using DTM for morphological identification& speciation or by rapid modern molecular techniques are useful for their prevention and cure.

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