

# Isolation and identification of fungal species in patients of recalcitrant tinea corporis and/ or tinea cruris attending tertiary care hospital in Karachi

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## Abstract

**Background** Tinea corporis and Tinea cruris is one of the chief infection seen in dermatology clinics affecting every age and gender. The rapidly rising prevalence of superficial fungal infection in our population warrants mycological analysis to establish any change in the causative dermatophytic specie responsible for ticking up the number of the cases.

**Objective** To isolate and identify the fungal species in patients of recalcitrant tinea corporis and/ or tinea cruris attending tertiary care hospital in Karachi.

**Methods** This Cross-sectional study was conducted in the Outpatient Department of Dermatology, Dow University Hospital, Karachi, Pakistan from 11th April 2022 till 10th October 2022. It comprised of 60 patients who had either received one complete course of oral antifungal treatment and had responded poorly to the therapy (identified on the basis of clinical response) and had a recurrence of disease after cure. The skin scrapings were collected from these patients and then microscopy and cultures were performed.

**Results** Out was 60 patients of Recalcitrant Tinea Corporis and /or T .cruris KOH mount was positive in 43 (71.6%) patients and the culture isolated and identified the causative species in 53 (88.3%) patients. T. Tonsurans account for about 33.3% of the cases followed by T. Mentagrophytes accounting for 15% of the patients. In 11.6 % of the patients isolated specie was T. Rubrum.

**Conclusion** Rise in the number of recalcitrant dermatophytosis could possibly due to change in the species causing dermatophytosis. Mycological analysis can help in identifying such species and facilitate in the treatment of these patients.

## Key words

Dermatophytes; Antifungals; Recalcitrant cases.

## Introduction

Superficial fungal infection of the skin and its appendages that is hair and nails are produced by a group of filamentous fungi having an ability to grow, colonize and affect the keratin.<sup>1</sup> The three genera-Trichophyton, Microsporum and Epidermophyton are responsible for the manifestation of dermatophytosis.<sup>2-3</sup> 20–25% of

world population is affected with superficial mycosis and it is one of the chief skin

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problem.<sup>4,5</sup> It can affect all age groups of people. They are classified depending on the clinical appearance into tinea corporis (ring worm of the skin of the trunk and limbs), tinea pedis (foot), tinea capitis (of the scalp), tinea cruris (groin), tinea barbae (bearded area of face and neck) tinea unguium (nail infection). The most prevalent clinical variant is tinea of glabrous skin followed by tinea of the groin, tinea of foot, and tinea unguium.<sup>6</sup> Of all the dermatophytic infection tinea corporis accounts for about 70% of the infection.<sup>7</sup> The prevalence of superficial fungal infection is though high all over the world but it is further higher in those people who are immunocompromised such as diabetics, cancers, organ transplant and AIDS patients and in regions which are more humid and densely populated and in low socioeconomic groups.<sup>8,9</sup>

They are generally diagnosed clinically by the clinical presence of advancing ring like lesion with active inflammatory margin and central clearing.<sup>10</sup> Diagnosis can also be easily confirmed by less invasive, quick and confirmatory investigation. Dermatophytes can be identified in the laboratory by performing fungal microscopy of skin scrapings on KOH (potassium hydroxide) and this is quick.<sup>10</sup> Fungal species can be isolated and identified by macroscopic features dermatophytic colonies (colour, growth rate, texture, etc.) when grown on special media, followed by a microscopic examination of conidia.<sup>11</sup> All over the World up to 80% of cases of Tinea corporis are caused by *Trichophyton rubrum* while in India it is responsible for around 88% of cases followed by *Trichophyton mentagrophytes*. The next most frequent organism is *Microsporum canis* both worldwide and in India followed by *Epidermophyton floccosum*.<sup>12,13</sup>

Various topical and systemic antifungals are being prescribed for the treatment of dermatophytosis including terbinafine,

fluconazole, itraconazole. Dermatophytosis are generally well treated with topical and systemic antifungals but recently resistance to antifungals has started emerging in dermatophytes.<sup>14</sup> In this study skin samples from recalcitrant tinea corporis and cruris patients were collected to isolate and identify dermatophytes by potassium hydroxide (KOH) and cultured on sabouraud dextrose agar (SDA).<sup>7</sup> Nowadays, it is being observed that there is not only a rise in the number of patients with superficial infections but at the same time rise in the number of patients who suffer relapse after the therapy is stopped or those who are not responding completely to the treatment. This study helped us to isolate and identify the causative fungal species in the recalcitrant cases and at the same time enables the physician to modify the treatment effectively. This study was helpful in knowing the recent trend of dermatophytic species in our population.

## **Methods**

This prospective cross sectional study was conducted in the Outpatient department, Department of Dermatology, Dow University Hospital from 11th April 2022 till 10th October 2022. This study was conducted after approval from Institutional Review Board, Dow University of Health Sciences. Total of 60 patients with recalcitrant Tinea corporis and/or Tinea cruris fulfilling the inclusion criteria were selected from the outpatient department, department of dermatology Dow University Hospital. Tinea corporis and cruris was diagnosed clinically as a ring shaped patches with active margins and central clearing.<sup>10</sup> Recalcitrant patients were those who had tinea corporis and/ or cruris showing partial or no improvement in symptoms after receiving (terbinafine 250 mg once daily for 4 weeks to 6 weeks or itraconazole 100 mg twice daily for 2 weeks) along with antifungal creams, or who

had developed lesions again after being cured of their previous infection.<sup>2</sup> Patients suffering from active infections (tuberculosis, septicemia), patients taking corticosteroids or other immunosuppressants, pregnant ladies and diabetics, organ transplant and cancer patients were excluded from the study.

All the selected patients were sent to the laboratory where the clinical pathologist after cleaning the site with 70% alcohol collected the sample by scraping with the scalpel from active borders of the lesions along with plucked hair follicles and then placed in sterile petri dish for microscopic examination and culture. Two drops of 10% KOH was placed on the glass slide to dissolve the keratin in the collected material. After placing the coverslip, slide was heated few seconds over the bunsen burner taking care only to warm the material and not to boil. The sample was then observed under the microscope to identify the causative agent and the observations were recorded. For the isolation of the dermatophytes from the skin scrappings sabouraud dextrose agar with gentamicin and dermatophyte test medium was used and incubated at 35°C and 25°C for up to 4 weeks. Gentamycin was added to inhibit the growth of saprophytic fungi and contaminating bacteria while permitting the growth of dermatophytic fungi. Reading of the culture plates were done daily for first week, then twice weekly for the next 3-4 weeks.<sup>15</sup> Dermatophytes are presumptively identified by gross morphology of their colonies and the production of alkaline metabolites which cause a change in the color in the medium from yellow to red.

Data was entered using Microsoft Excel 2013 (Microsoft Corporation, WA, USA) and analysed using SPSS version 22 software. Means±standard deviation were used to describe continuous variables such as age, whereas frequency and percentages were used to describe

categorical variables such as gender, marital status, clinical diagnosis, and the isolated species.

## Results

This was a 6 month cross-sectional study of recalcitrant cases of *T. corporis* and/ or *T. cruris* or both. The mean age of the patients at the time of presentation was 40.67±10.81. There were 21 males (35%) and 39 females (65%). Out of 60 patients 49 were married (81.7%) and 11 were unmarried (18.3%). 21 patients had *T. cruris*, 15 had *T. corporis* and 24 patients had both *T. cruris* and *T. corporis* (**Table 1**). Microscopy was conclusive in 43 patients (71.66%) and causative species of the fungus was isolated and identified via culture in 53 patients (88.3%) (**Table 2**). In 7 patients no growth was identified. *T. Tonsurans* was the most common fungal species isolated and account for about 33.3% of the cases followed by *T. Mentagrophytes* accounting for 15% of the patients. In 11.6% of the patients isolated species was *T. Rubrum* and 6.7% grew *M. Canis*.

**Table 1** Baseline characteristics of patients (n=60)

Characteristics	n (%)
Age Mean±SD,	40.67±10.812
Range (years)	(19-62)
Gender	
Male	21 (35.0)
Female	39 (65.0)
Marital Status	
Unmarried	11 (18.3)
Married	49 (81.7)
Occupation	
House wife	31 (51.7)
Student	7 (11.7)
Retired officer	1 (1.7)
Office Worker	11 (18.3)
Banker	2 (3.3)
Shop Keeper	5 (8.3)
Teacher	2 (3.4)
Domestic Help	1 (1.7)
Diagnosis	
<i>T. Cruris</i>	21 (35.0)
<i>T. Corporis</i>	15 (25.0)
<i>T. Corporis</i> and <i>T. Cruris</i>	24 (40.0)

**Table 2** Comparison of results obtained in the direct microscopic examination and culture.

	Grown on culture only	Microscopy and Culture both	None
	n (%)	n (%)	n (%)
T. Corporis	4(40.0)	10(23.3)	1(14.3)
T. Cruris	5(50.0)	16(37.2)	0(0)
T. Corporis and T.Cruris	1(10.0)	17(39.5)	6(85.7)

**Table 3** Species of superficial fungi isolated from samples.

Organism	T. Corporis n (%)	T. Cruris n (%)	T. Corporis and T. Cruris n (%)
T. Tonsurans	6 (40.0)	3(14.3)	11(45.7)
T. Mentagrophyte	2(13.0)	4(19.0)	3(12.5)
T. Rubrum	1(6.7)	4(19.0)	2(8.3)
M. Canis	1(6.7)	2(9.5)	1(4.2)
M.Violaceum	1(6.7)	1(4.8)	0(0)
M. Audionni	0(0)	2(9.5)	0(0)
M. Verrucosum	0(0)	0(0)	1(4.2)
Candida	0(0)	0(0)	1(4.2)
T. Tonsurans and candida	0(0)	1(4.8)	0(0)
T. Tonsurans and M.Audionni	0(0)	0(0)	1(4.2)
Microsporium canis and candida	0(0)	0(0)	1(4.2)
M. Violaceum and M. Audionni	1(6.7)	0(0)	0(0)
M. Violaceum and Aspergillus Niger	1(6.7)	0(0)	0(0)
T. Tonsurans and A.Niger	0(0)	0(0)	1(4.2)
E. Floccusum and A. Niger	0(0)	0(0)	1(4.2)
No Growth	2(13.3)	4(19.0)	1(4.2)

3.3% of the patients grew T. Violaceum and M. audionni in their culture. Candida and T. Verrucosum each was isolated in 1.7% of the patients. 11.7% of the patients grew two species together in which T. Tonsurans was isolated in 42.8% of the co infection. There was no growth in 11.7% of the patients (**Table 3**).

### Discussion

Superficial fungal infections was always simple to treat with a topical and systemic antifungal medications but recently this common infection of the skin has not only become rampant but its treatment has become a challenge for the physicians.<sup>16</sup> Recently there has been a considerable change in the worldwide distribution of the dermatophytes, leading to emergence of variable other species which were less frequently isolated previously.<sup>17</sup> There is a rise in the number of recalcitrant cases of tinea corporis and cruris likely due to the change in the causative species of dermatophytes or due to

microbiological resistance. Different geographical locations favour different infection patterns.<sup>18</sup> This study was helpful in knowing the recent change in the species of causative fungus that is leading to ticking up the number of recalcitrant cases. A study conducted in Karachi, Pakistan by Ambwani JK *et al.* on 39 patients showed that 27 (69.23%) grew T. rubrum, 4 (10.25%) had T. tonsurans, 3 (7.69%) grew T. Mentagrophytes, 2 (5.12%) isolated T. Verrucosum, candida was found in 3 (7.69%) patients.<sup>19</sup> Another study done in India isolated T. rubrum as the most common fungal species causing Tinea Corporis and T. cruris.<sup>20</sup> However, a study conducted on 124 patients of dermatophytosis diagnosed clinically from the dermatology outpatient department of AIIMS, New Delhi from June 2014 to July 2015 by Dabas *et al.* highlights the shift in epidemiology from T. rubrum to T. interdigitale.<sup>21</sup> Microscopy was positive in 74.1% (92/124) and 53.2% (66/124) grew on culture. The isolates include Trichophyton interdigitale (56%) followed by

Trichophyton tonsurans (25.7%), Trichophyton rubrum (7.5%), Trichophyton violaceum (4.5%), Microsporum gypseum (4.5%), and Trichophyton verrucosum (1.5%). In our study T. Tonsurans was responsible for about 33.3% of the cases followed by T. Mentagrophytes accounting for 15% of the patients. In 11.6 % of the patients isolated species was T. Rubrum.

## Conclusion

This study highlights that T. Tonsurans is emerging as a common isolate in recalcitrant cases of Tinea corporis and/or Tinea cruris. Seeing this changing trend warrants the mycological analysis of the causative species and even susceptibility testing to monitor treatment of patients and facilitate early detection of resistance to fungal agents.

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