Comprehensive analysis of *Trichophyton mentagrophytes* interdigitale complex from human and animal origin

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Objectives: Taxonomic delineation of etiologic agents responsible for recalcitrant dermatophytosis causing epidemic in India is still debated. The organism responsible for this epidemic was previously designated as *Trichophyton indotineae*, a clonal offshoot of *T. mentagrophytes*. 

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To evaluate the accurate identity of the agent causing the epidemic, we performed multigene sequence analysis of Trichophyton species isolated from human and animal origins.

Methods: The clinical isolates of T. mentagrophytes/interdigitale complex (n = 213) from PGIMER, and six isolates of animal origin (zooine) from IVRI were included in the study. Seven genomic loci including internal transcribed spacer (ITS) region (n = 213), translational elongation factor (TEF 1-a) (n = 69), patellar beta tubulin (BT) (n = 69), large ribosomal subunit (LSU) (n = 54), calmodulin (CAL) (n = 27), high mobility group transcription factor (HMG) (n = 17), and α-box (n = 17) were amplified by polymerase chain reaction (PCR). Phylogenetic tree was constructed by neighbor-joining method using ours and sequences retrieved from GenBank. Trichophyton genotyping was used in our group due to its high divergence. The identity labeling was validated as per the phylogenetic analysis of ITS gene in the present study (ITS gene (n = 213), TEF1-a (n = 184), BT (n = 52), LSU (n = 69) and HMG transcription factor (n = 50)).

Results: Phylogenetic analysis of ITS, TEF 1-a, LSU gene revealed that except for one isolate (PGI-IVRI B24-A) of animal origin belonged to ITS genotype III and not all isolates (human (n = 213) and animal origin (n = 6)) belonged to the same cluster, i.e., T. mentagrophytes/ITS genotype VIII. The phylogenetic tree constructed based on the ITS gene (n = 39) also clustered all our isolates together and isolates belonging to T. mentagrophytes ITS genotype IV, VIII, and T. interdigitale and one isolate (PGI-IVRI B24-A) formed a separate cluster and did not coalesce with other genotypes. Whereas CAL gene clustered all our isolates (n = 26) together except PGI-IVRI B24-A isolate which grouped with T. mentagrophytes type VII. Further HMG analysis reveals that all the isolated Trichophyton species from humans (n = 11) and animals (n = 5) contained HMG transcription factor but lacked α-box genes. One animal isolate (PGI-IVRI B24-A) consisted of both HMG transcription factor and α-box genes.

The accurate identity of the isolates was based on our phylogenetic analysis (ITS gene). Based on the analysis of the 374 ITS sequences deposited in the GenBank database, 107 sequences were re-clustered as T. mentagrophytes was named T. interdigitale or two varra. Similarly, 184, 52, 69, and 502 sequences of TEF1-a, BT, LSU, CAL, and HMG transcription factor deposited in the GenBank have 6, 25, 25, 5, and 7 sequences were unlabeled, respectively. Further, for the identical isolates (n = 6), we found multiple accession numbers with different labels.

Conclusion: In this study, for the first time we isolated Indian ITS genotype VIII from animal. Isolation of T. mentagrophytes type III from animal but none among humans indicates its niche among animals. Adding to the confusion, nondiscriminate naming for these dermatophytes in the public database has created confusion in using appropriate species designation.

Figure 1. Phylogenetic tree based on ITS sequences (representative isolates) Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The evolutionary distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA X.
Figure 2. Phylogenetic tree based on ZEF 1-α sequences Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates). The evolutionary distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA X.