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Comparative evaluation of Dermatophyte-PCR kit, with conventional methods for rapid detection of dermatophytes and identification of *Trichophyton rubrum* in nail specimens

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Introduction

Onychomycosis is a common and persistent fungal infection caused chiefly by *Trichophyton rubrum* and *Trichophyton interdigitale.* Diagnosis has relied on a combination of microscopy and culture based techniques; however, microscopy lack sensitivity, specificity and their growth may take to weeks. A fast and accurate detection and identification of the causal agent in onychomycosis is essential, since requires long-term systemic antifungal treatment. Recently, many PCR assays have been developed to improve onychomycosis diagnosis. In this study, a commercially available multiplex PCR kit, which combines pan-demartophytes PCR with a *T.rubrum*-specific PCR was compared with KOH microscopy and culture isolation for diagnosis of dermatophytes in nail specimens.

Materials and Methods

Nail specimens (n=130) were prospectively collected from patients with clinically suspected onychomycosis and were studied by KOH microscopy, cultures and multiplex PCR kit (Dermatophyte PCR kit, Statens Serum Institut, SSI Diagnostica, Denmark) in a mycology reference laboratory. Fungal DNA was easily and rapidly (15 min) extracted using a commercial kit. The multiplex PCR using novel primers targeting the pan-dermatophyte-specific sequence of the chitin synthase 1 gene (CHS1) for detecting dermatophytes generally and ITS2 (internal transcribed spacer) for detecting *T. rubrum* was performed . The test was completed within 5 h.

Results

More samples were positive by PCR (63/130, 49%) than by culture (36/130, 28%). Only 2 culture positive specimens (*T. rubrum* n=1, *T. interdigitale* n=1) were not detected by PCR, possibly due to the small quantity of the examined material. *T.rubrum* could be identified in 45% (26/58) of cases in which the culture results were negative. Since the majority of PCR positive but culture negative samples were positive in microscopic examinations (19/27), the increased sensitivity was not due to contamination (Table 1). From 11 specimens diagnosed by conventional methods as non-dermatophyte or *Candida* species, one gave a positive PCR-result (*T. rubrum*) (Fig. 1). The sensitivity, specificity, positive predictive value, and negative predictive value of the multiplex PCR was 94%, 69%, 54% and 97%, respectively, when confirmed by positive culture.

References

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Results

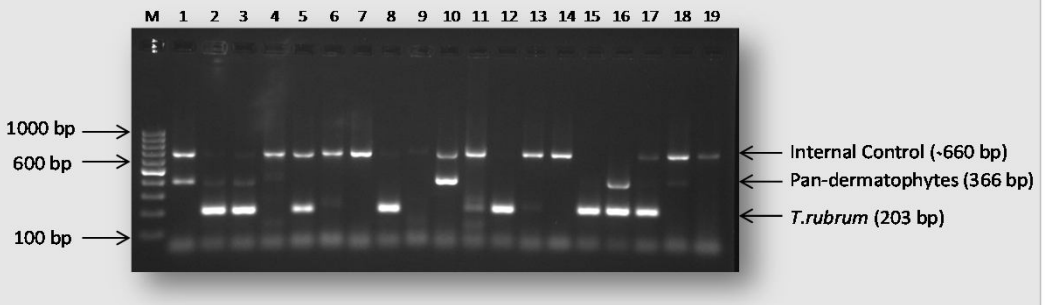

Fig. 1. Example of *Trichophyton rubrum*-specifik and pan-dermatophyte multiplex PCR product analysis. Lanes: M, Marker (100 bp DNA ladder); 1, dermatophyte genomic DNA (control 1); 2, *T. rubrum* genomic DNA (control 2); 3 to 18, results of multiplex PCR performed for DNA extracted directly from nail specimens diagnosed by culture and microscopy as *T. rubrum* (Lane 3, 5, 6, 8, 11, 12, 13, 15, 16, 17) ; negative (Lane 4, 7, 9, 14); *Trichophyton interdigitale* (Lane 10, 18), and 19, negative control.

Table 1. Microscopy and culture results from nail specimens with suspected onychomycosis in relation to positive or negative PCR results.

	Positive PCR							
	T.rubrum		Dermatophyte		Negative PCR		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Microscopy								
Positive	44	(%65.7)	5	(%7.5)	18	(%26.9)	67	(%51.5)
Negative	14	(%22.2)	0	(%0,0)	49	(%77.8)	63	(%48.5)
Culture								
Positive	32	(%55.2)	2	(%5.6)	2	(%5.6)	36	(%27.7)
Negative	26	(%27.7)	3	(%3.2)	65	(%69.1)	94	(%72.3)

% refers to total number of specimens, n=130

Conclusions

The multiplex-PCR method applied directly on nail specimens is simple, fast and reliable. Therefore, a polyphasic approach to identification that combines conventional method (morphologic identification) and multiplex-PCR method will ensure the greatest success in the management of patients with suspected onychomycosis.