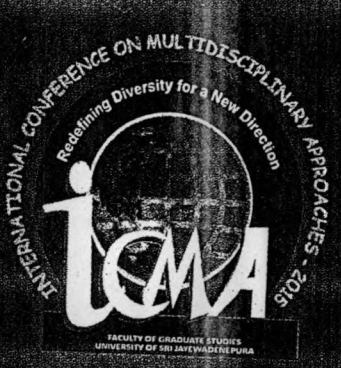

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USE OF A MULTIPLEX PCR TO IDENTIFY *CANDIDA SPECIES* IN CONCENTRATED ORAL RINSE SAMPLES OF PATIENTS WITH DIABETES

M.K.A. Sampath[1], T.D.C.P. Gunasekera[1], J. Kottahachchi[1], K.A.A.Dilhari[1], U.Bulugahapitiya[2], S.S.N. Fernando[1], M. M. Weerasekera[1]

[1] Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura
[2] Endocrinology unit,Colombo South Teaching Hospital.

Oral candida infections are most frequently observed in patients withdiabetes.As diabetes has become the number one non communicable disease in Sri Lanka, oral candida infections are an emerging problem. Although Candida albicans is the predominant pathogen in oral candidiasis multiple Candida species involvement is common. Hence it is important to develop rapid, sensitive and specific molecular based methods to identify multiple Candida species in clinical specimens.

The aims of this study were to optimizeand apply a multiplex PCR to identify four important Candida species, namely *C.albicans, C.parapsilosis, C.glabrata* and *C.tropicalis* in concentrated oral rinse samples of patients with type 11 diabetes. The performance of multiplex PCR was compared with phenotypic identification.

A multiplex PCR was optimized to identify *C.albicans, C.parapsilosis, C.glabrata* and *C.tropicalis* in concentrated oral rinse samples of patients with diabetes, attending the Endocrinology clinic at Colombo South Teaching hospital. Multiplex PCR wasoptimized using a common reverse primer, ITS4 and four species specific primers targeting ITS 1 and ITS2 regions of yeast genome (primer CA, CT, CP, and CGL respectively). Optimized multiplex PCR was applied to identify four different *candida species* in 20 clinical samples and the results were compared with results of phenotypic identification for Candida ie; colony characteristics, germ tube test, sugar assimilation and clamydospore formation. Further antifungal susceptibility test was performed using disk diffusion method (NCCLS guideline M 44) for colonized patients.

Out of the 20 oral rinse samples, 10 were culture positive. However, only 8 samples were colonized (> 600 CFU/ml) with *Candida species*. Out of these 8 patients, multiple *Candida species* were identified in 5 patients, where all of them had *C. albicans*alone with *either C. Parapsilosis*or *C. tropicalis*. Three patientshad only *Candida albicans*.The 20 samples tested with multiplex PCR, 14 were positive for *Candida spp*. All 14 contained *C. albicans*with 12 being positive for multiple *Candida spp*. including *C. parapsilosis* (10/20), *C. tropicalis* (4/20) and *C. glabrata* (4/20).

Established multiplex PCR is found to be rapid, sensitive and more specific than conventional culture method in identifying multiple *candida species* in oral rinse samples.