ABSTRACT

Background
Candida species are endogenous fungus causing both mucosal and invasive infections. With the increasing rate of infections due to non- Candida albicans and varying susceptibilities to commonly used empirical antifungal agents like fluconazole; early and accurate species identification would help the clinician in befitting therapeutic management. There is a need to search for new products with effective antifungal abilities, Due to the adverse side effects of existing medications, increasing emergence of resistance to conventional antifungal agents, and the formation of biofilms in medical devices and tissues So the interest in natural products secreted by a fungus itself as an antimicrobial agent has evaluated in the present study. This study was undertaken to know the rate of candidiasis, antifungal susceptibility, virulence factors like biofilm, proteinase, phospholipase and evaluate the killing capacity of environmental killer toxin Wickerhamomyces anomalous against pathogenic Candida species under optimum conditions.

Materials and methods
The study was conducted in the Department of Microbiology, Travancore Medical College and Hospital, Kollam, Kerala. The Institutional Ethical Committee has approved the protocols used in this study. A total of 336 Candida species isolated from various clinical samples were included in the study. Candida identification, antifungal susceptibility, biofilm formation, proteinase and phospholipase action were done by standard procedures. The environmental yeast killer toxin Wickerhamomyces anomalous was purified and the killer toxin assay against Candida species was done by standard procedures.

Results
The predominant Candida species were Candida albicans (31%), but the emergence of non Candida albicans also noted with 69%. The major clinical condition of candidiasis was vulvovaginitis associated with pregnancy (40%) was followed by candiduria (21%) and candidemia (12%). The pre-disposing condition related to candidemia was patients undergoing chemotherapy associated with malignancy (23%).
The predominant virulence factor was found to be biofilm production (68%) in Candida isolates. Total antifungal resistance was 117(35%) with fluconazole resistance in Candida albicans 13(13%) and in non-Candida albicans were 50(21%). All the candidemia isolates tested for caspofungin were sensitive with MIC ≤2 µg/ml in blood isolates.

The characterization of environmental yeast killer toxin W. anomalus has revealed that it is a glycoprotein of having 42.6kDa. The killer activity of W. anomalus was higher at low temperature and at low pH, but as temperature and pH increased killer activity decreased. Killer activity was highest at pH 5.5 with temperatures 25\(^0\) C and lowest at pH 6 and 30\(^0\)C.

W. anomalus killer toxin assay against the Candida isolates showed 292(87%) were sensitive to the killer toxin. There was no statistically significant difference (p value >0.05) between biofilm producing Candida albicans and non-Candida albicans with respect to killer toxin action. Among the antifungal resistant isolates of Candida 105(91%) were sensitive to W. anomalus killer toxin and 197(90%) antifungal sensitive isolates of Candida were sensitive to W. anomalus killer toxin. This proves that antifungal resistance is also not interfering with killer toxin action (p value is >0.05).

**Conclusion**

The study emphasizes the need for rapid and precise speciation of Candida isolates for effective treatment and management strategies. The present study also is in favor of the need for periodic surveillance of the antifungal susceptibility pattern of the prevalent Candida species. Antifungal susceptibility method outlined that Candida isolates were more susceptible to amphotericin B and flucytosine to that of azoles with increase in resistance to fluconazole is a matter of great unease as it is the most commonly used azoles for the treatment. Voriconazole exhibited the greatest activity in our study. The virulence of Candida species is attributed not to a single factor but to a combination of several factors like proteinase, phospholipase and biofilm production.
The study indicates that multiple yeast killer toxins are produced by different isolates of the same species of yeast. Our study proved the antifungal ability of environmental yeast killer protein of *W. anomalus* and this resource can be used for alternative treatment of candidiasis with further assessments. *W. anomalus* mycocin demonstrated a broad spectrum of inhibitory action against *Candida* species. The growth inhibition of *W. anomalus* mycocin against *Candida* species should be further explored for therapeutic potentials against candidiasis.

**Keywords:** *Candida albicans, non-* *Candida albicans,* antifungal susceptibility, biofilm, proteinase, phospholipase, yeast killer toxin, *Wickerhamomyces anomalous*