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An overexpression screen identifies Csa25 as a novel cellular morphogenesis regulator in the human fungal pathogen *Candida albicans*

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Objective: Morphological plasticity is one of the key attributes of microbial pathogens contributing to the successful establishment of infection in host tissues. *Candida albicans*, an opportunistic human fungal pathogen, lives as a commensal in the gut, skin, and genitourinary tracts of most healthy individuals. The budding yeast form helps it to disseminate easily in the host system, and the filamentous form (hypha and pseudohypha) is believed to invade the host tissue. Strikingly, alterations of gene expression that block cell cycle progression at different stages additionally lead to aberrant cellular morphology in *C. albicans*. While various morphological states of *C. albicans* have been well-studied, the search for key players bringing about these changes is far from complete. This is supported by the fact that ~70% of the *C. albicans* proteome remains functionally uncharacterized. Thus, the primary objective of our study was to identify novel regulators contributing to cellular morphogenesis in *C. albicans*.

Method: In our current study, we screened an overexpression library of *C. albicans* ORFeome generated to identify novel regulators contributing to chromosome stability (CSA) in *C. albicans*. The screen involved overexpression of each gene using a tetracycline-inducible promoter for a duration of 12 h, followed by microscopy-based observations to identify associated aberrant cellular morphologies.

Results: Screening of overexpression library of the *C. albicans* ORFeome identified 14 unique Candidate genes from 1389 genes screened. While the functions of half of them have been verified in *C. albicans*, the remaining seven genes are not functionally characterized. Each of the seven uncharacterized genes was predicted to be non-essential for viability in *C. albicans*. Bioinformatic analysis predicts one of these proteins, Csa25, to be carrying a point centromere-specific kinetochore protein Ndc10-like DNA-binding domain at its N-terminus spanning over a region of 273 amino acids. Sub-cellular localization indicates this protein to be present throughout the nucleus at all stages of the cell cycle. Strikingly, overexpression of this protein led to yeast cells forming chains connected by septa, as visualized by calcofluor staining, without hampering nuclear segregation. In addition, a large proportion of cells overexpressing Csa25 were unable to exhibit hyphal morphology when subjected to hyphae-inducing conditions.

Conclusion: In conclusion, our study identified Csa25 as a novel morphogenesis regulator involved in the negative regulation of yeast-hyphae transition in *C. albicans*. Further studies based on host-pathogen interaction will identify the critical role of Csa25 in the pathobiology of *C. albicans* and its survival in host-specific niches.

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A tele of fungal burden in chronic suppurative otitis media (CSOM) patients of a tertiary care center of Nepal

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Objectives: The study was designed to find out the fungal etiological agents in chronic suppurative otitis media (CSOM) patients attending the tertiary care center of Nepal.

Methods: The laboratory-rooted study was performed at the Department of Clinical Microbiology. Specimen was collected in the ENT and Head and Neck Surgery Department of Tribhuvan University Teaching Hospital (TUTH), Nepal from February 2016 to July 2016. All clinical specimens were collected from hospitalized as well as outdoor patients having CSOM. Specimens were processed according to standard methodology. A total of 117 patients having CSOM were confirmed cases by the otolaryngologists and their 123 specimens were included in the study. Ear discharge was collected using sterile swab sticks which were labeled and sent to the laboratory for potassium hydroxide (KOH) mount and fungal culture studies.

Results: A total of 123 specimens were collected and processed. Distribution of patients according to the site among the total patients (n = 117), 69 (59.0%) were specimens from the left ear, 42 (35.9%) right ear, and 6 (5.1%) from both ears (bilateral) (Table 1). The 19-30 years age group was highest (34.1%) and followed by 31-50 years having 23.6%. Occupationally students were higher in number (29.9%) and it was followed by housewives (27.4%). A total of 47.8% of cases are from Kathmandu and remain from different regions of Nepal. Out of 123 specimens, 23 (18.7%) were found KOH mount positive (Table 2). The distribution of fungal isolates is as follows—among total isolates *Aspergillus flavus* 7, *A. fumigatus* 6, *Acremonium* 3, *Candida albicans* 2, *Penicillium* 2 *A. niger* 1, *C. krusei* 1, *C. tropicalis* 1, *Curvularia* 1, *Fusarium* 1, *Mucor* 1, and *Syncephalastrum racemosum* 1 (Table 3).

Conclusion: The prevalence of fungi in CSOM patients was quite high (21.9%). This observation was different from the study of India conducted by Kumar et al. (15%) and in contrast with another researcher in Singapore, Loy et al. (8.8%). Aslam et al. from Pakistan study revealed only 2.1% and Khwakhali et al. study from Nepal estimated about 1.94% of the Nepalese population suffer from a serious fungal infection annually (commonly in HIV/AIDS and immunocompromised hosts) which are diluted by our findings. The possible reason may be due to location, temperature, negligence on mycological complications, and their treatment in Nepal. Treatment of CSOM should be based on the result of fungal culture. CSOM cases are found in all age groups (2-80 years) with various health statuses, different occupations, and in dispersed regions of Nepal. Phenotyping identification is cumbersome and have risk of infections which increases the chance of applying genotyping technique will be beneficial. Antifungal susceptibility testing should be mandatory since it helps in improving clinical outcomes by optimization of antifungal practices. Many CSOM patients complained that they were not cured even long time of use of antibacterial drugs. It clears that fungal etiological agents can't be neglected. If I am not wrong, Nepal has no separate designated mycology laboratory. There is also a lack of funding for clinical fungal studies and their awareness regarding fungal pathogens.