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Species distribution and biofilm profile of *Candida* isolated from clinical specimens at a tertiary care hospital in India

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Introduction: The epidemiology of invasive candidiasis (IC) is dynamically changing, given the increasing population of susceptible hosts, use of indwelling medical devices (IMD), and environmental factors. The presence of an IMD is one of the most important risk factors for persistent infection due to the possibility of biofilm formation. The biofilm cells are significantly less susceptible to antifungal drugs and are able to evade the host immune system, serving as a nidus for reinfections.

Objectives:

1) To determine the species distribution of *Candida* isolated from clinical specimens of hospitalized patients.
2) To evaluate biofilm formation by clinically significant and colonizing isolates of *Candida* species recovered from clinical specimens.

Methods: A total of 100 *Candida* isolates from patients with suspected invasive candidiasis were tested for the production of biofilm. Based on clinical history, 62% of the isolates were found to be clinically significant, while 38% represented commensals or colonizers. Species identification was done on the basis of germ tube test, CHROMagar, Dalmau plate technique, and carbohydrate fermentation and assimilation tests, and VITEK 2. Four isolates that failed to be identified by conventional methods were subjected to MALDI-TOF MS. Biofilm production was detected and graded by visual (test tube) and spectrophotometric (microtiter plate) methods.

Results: Non-*albicans Candida* (NAC) were the predominant clinically relevant isolates recovered from cases of IC (71%), while *C. albicans* was most commonly associated with colonization (68.4%). Among the NAC isolates, *C. tropicalis* was the most common isolate (23%) followed by *C. glabrata* (11%), *C. krusei* (8%), *C. parapsilosis* (6%), *C. lusitaniae* (2%), *C. kefyr* (2%), *C. rugosa* (2%), *C. guilliermondii* (1%), and *C. famata* (1%) (Fig. 1). A total of 55% of the *Candida* isolates produced biofilm. Biofilm positivity in clinically relevant isolates was found to be significantly higher than commensals/colonizers ($P < .05$). Biofilm positive *Candida* spp. were most commonly isolated from urine (84.6%) followed by blood (67.8%). Biofilm production by NAC (69%) was found to be significantly higher than *C. albicans* (31%) ($P < .05$). Majority of the biofilm positive isolates produced Grade 2 (moderate) biofilm (36.4%). *C. tropicalis* accounted for maximum biofilm production comprising 20% of Grade 4, 53.8% of Grade 3, and 50% of Grade 2 biofilm (Fig. 2). There was 72.7% concordance between the two methods in grading of biofilm. Spectrophotometric method was found to be more sensitive than a visual method for the detection of biofilm.

Conclusion: Our study demonstrated a paradigm shift from *C. albicans* to NAC with the isolation of *C. tropicalis* from a large number of cases, highlighting the growing importance of this pathogen. The knowledge about local epidemiological trends of *Candida* spp. is important to guide therapeutic choices. Moreover, clinically relevant *Candida* spp. were found to possess a greater ability to produce biofilms than commensals or colonizers. These findings are unique as previous studies haven't differentiated between biofilms formed by commensal *Candida* populations and those related to infections. This study highlights that biofilm production should be considered a relevant biologic variable while treating patients with invasive candidiasis, particularly those who fail to respond to antifungal therapy.