

<https://doi.org/10.33472/AFJBS.6.6.2024.5629-5632>



## African Journal of Biological Sciences



# Comparison of Corn meal broth method and Dalmau plate method for demonstration of Chlamydo spores in *Candida albicans*.

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### Article History

Volume 6, Issue 6, 2024

Received: 15 JAN 2024

Accepted: 22 April 2024

[doi.org/10.33472/AFJBS.6.6.2024.5629-5632](https://doi.org/10.33472/AFJBS.6.6.2024.5629-5632)

### ABSTRACT

*Candida* occurs as normal commensal in healthy individuals, and is usually present on the skin and mucosal surfaces of the oral cavity, gastrointestinal and vagina and urogenital tracts. However it can cause a wide variety of infections in immunosuppressed patients like those on broad - spectrum antibiotics, steroids, immunosuppressive drugs, diabetes mellitus, AIDS, Malignancy, Neutropenia. Majority of infections caused by *Candida albicans*. Non- *C. albicans* *Candida* (NCAC) species are emerging as important pathogen in humans. Our study included 44 clinical isolates which showed gram positive budding yeasts cells with or without pseudohyphae and Germ tube positive. In this study we compared liquid media and slide culture for the rapid production of chlamydo spores. Among 44 *C. albicans*, with broth method 38 (86.6%) showed single terminal chlamydo spores at 16 hours of incubation. Standard dalmau plate method showed budding yeast cells with pseudohyphae without chlamydo spores at 16 hours but after 72 hours all the isolates showed characteristic chlamydo spores.

**Key words**-Chlamydo spore, corn meal broth, dalmau plate

### Introduction:

*Candida* causes infection in all communities worldwide. *C. albicans* being the most common fungal pathogen in humans. It occurs as a commensal on mucosal surfaces in many healthy individuals [1]. Majority of infections caused by *Candida albicans*. Non- *C. albicans* *Candida* (NCAC) species are emerging as important pathogen in humans [2, 4, 5,6,7]. Any alterations in host or environment leads to overgrowth of fungus and infection to the host. In many cases of impaired local or general health, *C. albicans* can assume a pathogenic role giving rise to acute and chronic candida infections like thrush, atrophic glossitis, angular stomatitis, leukoplakic lesions, genital infections, esophageal candidiasis, systemic candidiasis [5, 6]. Production of chlamydo spore and

germ tube is a low cost diagnostic tool for *Candida albicans* identification. Chlamydo spores are grown by standard Dalmau's technique on Cornmeal agar (CMA) with 1% tween 80 after 72 hours of incubation [4]. Several modifications available to hasten the process of chlamydo spore production. The study was designed to compare the duration required for chlamydo spore production by Dalmau's method and cornmeal broth (CMB) method.

## Materials and methods

Study Design–Prospective cross sectional study

Study period– March 2023 to August 2023

Study centre– Gulbarga Institute of Medical Sciences

Inclusion criteria: 1. Patients attending OPD And IPD and showed presence of budding yeast cells on Grams stain, Germ tube positive and who were immunocompromised.

Exclusion criteria–Contaminated Samples,Patients already on antifungal therapy, Normal Commensals

Methodology: 44 isolates of candida from various isolates of urine, blood, sputum, endotracheal aspirate, vaginal swabs that showed germ tube positive from patients attending Gulbarga Institute of Medical Sciences were chosen for the study. The isolates were grown on potato dextrose agar for 48 hours .Corn Meal broth was prepared by refrigerating corn meal agar in distill water for overnight and then filtering using whatmann filter paper. 5% milk was then added and autoclaved at 121 C for 15 min at 15 lbs pressure and dispensed 1 ml of broth in test tubes .one colony from the Potato dextrose agar was inoculated in it and incubated at 28 C for 16 hours [8, 9] . Wet mount was prepared and looked for chlamydo spore formation [3] .All 44 isolates were streaked on SDA agar and incubated at 42 C to differentiate between *C. albicans* and *C. dublinensis*. For dalmau technique, by slide method 1 cm \* 1 cm agar was cut, placed on glass slide and streaked with the isolate at 4 edges and covered with cover slip incubated at 28 C for 72 hours and looked for chlamydo spore formation Majority of infections caused by *Candida albicans* .Non- *C. albicans* *Candida* (NCAC) species are emerging as important pathogen in humans [10].

Plate culture was done using dalmou method and incubated at 28 C for 72 hours and looked for chlamydo spore formation.Majority of infections caused by *Candida albicans* .Non- *C. albicans* *Candida* (NCAC) species are emerging as important pathogen in humans [11]

**Results:** Among 44 *C. albicans*, with broth method 38 (86.6%) showed single terminal chlamydo spores and 6 isolates showed budding yeast cells with pseudohyphae without chlamydo spores at 16 hours of incubation. Standard plate method showed budding yeast cells with pseudohyphae without chlamydo spores at 16 hours but after 72 hours all the isolates showed characteristic chlamydo spores confirming the identity.

**Discussion:** In the present study ,Isolates showing budding yeasts cells in routine urine , blood , vaginal swab, endotracheal swab was inoculated in SDA media for fungal growth at 37°C for 2 days. Positive fungal growth cases were undergone for germ tube test to identify *C. albicans* at 42°C. We found two types of germ tubes. First, Germ tubes were cylindrical outgrowths rose from blastospores and grown continuously by extension whereas second one is the filamentous outgrowths of *Candida tropicalis* which are known as pseudohyphae those were budding cells that remain attached to the blastospores and may elongate. When pseudophyphae elongate, they may resemble germ tubes. Differentiation between the two depends on the presence of a constriction at

the junction between the filamentous outgrowth and the mother blastospore in a pseudohypha and the absence of such a constriction in a germ tube.

In our study confirmatory test of identification of *C. albicans* was carried out by formation of chlamydospore

In this study we compared liquid media and slide culture for the rapid production of chlamydospores i.e., corn meal broth +5% milk. The quantification of chlamydospore was done at 16 hours. The result showed that the corn meal broth + 5% milk gave rapid and more numbers of chlamydospores as compared to slide culture. Some authors have suggested that the time required to produce them with standard method is 48-72 hours in rice meal agar and tensoactive agents. This time can be reduced or sorted using liquid media such as corn meal broth and diary supplements.

Corn meal agar stimulates sporulation of *C. albicans*, and is useful in suppressing certain other fungal growth, while milk enhances the formation of chlamydospore by 21.4-95.5%.

**Conclusion:** Broth method was easy to perform, less laborious, results were obtained in 16 hours and was easy to observe under microscope. Broth method can be easily adapted as routine diagnostic laboratory procedure for germ tube positive *Candida* isolates.



Figure 1: Wet mount for chlamydospores from Corn meal broth with 5% milk at 16 hours

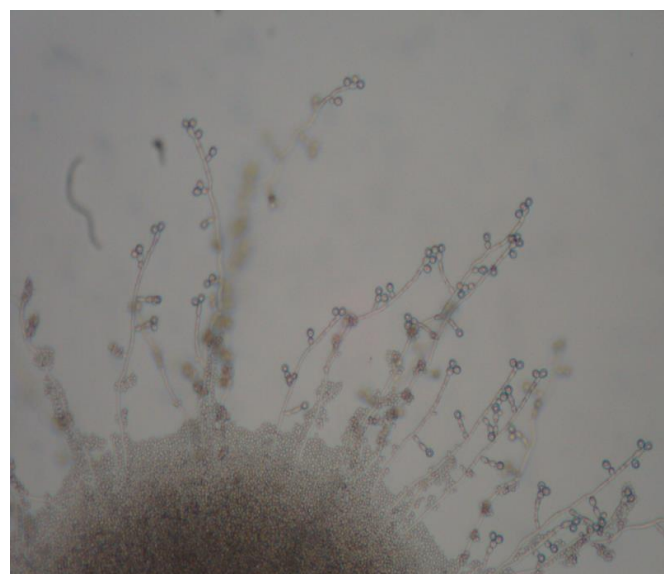


Figure 2: (Microscopy 10x ) Chlamydospore formation from corn meal agar by Dalmou technique

**Acknowledgement**–Technical staff of Microbiology Department and administrative staff of GIMS Gulbarga

**Financial Support and sponsorship:** Nil

**Conflicts of interest:** There are no conflicts of interest

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