

**EVALUATION OF CONGO RED AGAR FOR DETECTION OF BIOFILM PRODUCTION BY VARIOUS CLINICAL CANDIDA ISOLATES**Naveen Saxena<sup>1</sup>, Deepak Maheshwari<sup>2</sup>, Divya Dadhich<sup>3</sup>, Savita Singh<sup>4</sup>**HOW TO CITE THIS ARTICLE:**

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**ABSTRACT: BACKGROUND:** Biofilm is one of the known virulence factors of *Candida*, an important pathogen and commensal. Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are highly resistant to antimicrobial agents. Early detection of biofilm production may be useful for clinical decision because of its suggestive property for potential pathogenic capacity of *Candida* isolates. There are various methods to detect biofilm production like Tissue Culture Plate (TCP), Tube method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors, and fluorescent microscopic examination. **OBJECTIVE:** This study was conducted to evaluate Congo Red Agar method for the detection of biofilms. **METHOD:** The study was carried out at the Department of Microbiology, Government Medical College, Kota (Rajasthan) from April 2012 to June 2013. A total of 120 clinical *Candida* isolates were subjected to biofilm detection method. Isolates were identified by standard microbiological procedures. Biofilm detection was tested by CRA method. **RESULTS:** From the total of 120 clinical *Candida* isolates, CRA method detected 38.33% as biofilm positive and 61.66% cases as biofilm negative. Out of total biofilm positive *Candida*, 21.73% were strong biofilm producers and 78.27% were weak biofilm producers. **CONCLUSION:** We can conclude from our study that the CRA method is a quantitative and reliable method for the detection of biofilm forming microorganisms and it can be recommended as a general screening method for detection of biofilm producing *Candida* in laboratories.

**KEYWORDS:** biofilms; *Candida*; Congo red agar.

**INTRODUCTION:** Microorganisms universally attach to surfaces and produce extracellular polysaccharides, resulting in formation of biofilm.<sup>1</sup> Biofilms are defined as microbial derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. They are embedded in a matrix of extracellular polymeric substances (EPS) they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. Within a biofilm, microorganisms communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing. Availability of key nutrients, chemotaxis towards surface, surface adhesins and presence of surfactants are some factors which influence biofilm formation.<sup>2</sup> Microorganisms growing in a biofilm are intrinsically more resistant to antimicrobial agents than planktonic cells. High antimicrobial concentrations are required to inactivate organisms growing in a biofilm, as antimicrobial resistance can increase to 1,000 fold.<sup>3</sup>

*Candida* is the major fungal pathogen of humans causing a variety of afflictions ranging from superficial mucosal diseases to deep seated mycoses. One of the important factors contributing to the virulence of *Candida* is the formation of "biofilm".<sup>4</sup> Eradication of biofilm is difficult and biofilm producing *Candida* species are significantly less susceptible to antimicrobial agents.<sup>5</sup> With the emergence of biofilm associated diseases, there are considerable diagnostic problems for the clinical

## ORIGINAL ARTICLE

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laboratory, decreased antimicrobial susceptibility, false negative cultures, visible but not cultivable organisms or inappropriate specimen.<sup>1</sup> The determination of biofilm production in *Candida* sp. may be important for the management of invasive infections.<sup>6</sup> There are various methods to detect biofilm production. These include the Tissue Culture Plate (TCP),<sup>7</sup> Tube method (TM),<sup>8</sup> Congo Red Agar method (CRA),<sup>9</sup> bioluminescent assay,<sup>10</sup> piezoelectric sensors<sup>11</sup> and fluorescent microscopic examination.<sup>12</sup>

We screened 120 *Candida* sp. by Congo red agar method, which could be used in a routine clinical laboratory, for determining their ability to form biofilm.

**OBJECTIVES:** The study was conducted to detect biofilm formation by different *Candida* species isolated from various mucocutaneous clinical specimens by Congo red agar method.

**MATERIAL AND METHOD:** After taking ethical clearance from: Institutional Ethics Committee the study was conducted at the Department of Microbiology, Government Medical College and M.B.S hospital, Kota (Rajasthan) from April 2012 to July 2013. A total of 120 *Candida* specimens were collected from patients admitted in different wards of medical college and M.B.S hospital, Kota during the period of study. Mucocutaneous clinical samples were included from oral thrush vaginitis, keratitis and mucocutaneous candidiasis.

In this study, a total of 120 non-repetitive *Candida* isolates including *C. albicans* (51), *C. glabrata* (24), *C. krusei* (14), *C. Parapsilosis* (7), *C. tropicalis* (19), *C. guilliermondii* (2), *C. dubliniensis* (15) and *C. kefyr* (2) were used. They were isolated from oral thrush (60), vaginitis (36), keratitis (15) and chronic mucocutaneous candidiasis (9). The identification of *Candida* species was conducted by using conventional methods (germ tube formation, microscopic morphology in Cornmeal-Tween 80 agar, carbohydrate fermentation and assimilation tests). Colony morphology was also observed on HiCrome *Candida* Differential Agar. Prior to being tested, all strains were subcultured at least twice on Sabouraud dextrose agar (SDA) to ensure viability and purity.

**Congo red agar method (CRA):** Biofilm production was determined by using a modification of the test established for coagulase-negative *Staphylococci*<sup>9</sup> which requires the use of a specially prepared solid medium-Brain heart infusion broth (BHI) supplemented with glucose and Congo red. The medium was composed of BHI (37 gms/L), glucose (80 gms/L), agar no.1 (10 gms/L) and congo red stain (0.8 gms/L). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 min, separately from other medium constituents and was then added when the agar had cooled to 55°C. Plates were inoculated and incubated aerobically for 24 to 48 h at 37°C. Positive result was indicated by dark red colonies. Weak biofilm producers usually remained pink, though occasional darkening at the centres of colonies was observed. Biofilm negative strains produced white or very light pink coloured colonies. The experiment was performed in triplicate and repeated three times. *Candida albicans* ATCC 90028 and *C. parapsilosis* ATCC 96142 served as controls for biofilm production.

**RESULT:** Out of the 120 strains of *Candida* isolated from various clinical samples, 60(50%), 36(30.0%), 15(12.5%) and 09(7.5%) were from oral thrush, vaginitis, keratitis and chronic mucocutaneous cases respectively. Various *Candida* species recovered from clinical samples were *C.*

## ORIGINAL ARTICLE

albicans (42.5%), followed by *C. tropicalis* (15.8%), *C. dubliniensis* (12.5%), *C. krusei* (11.6%), *C. glabrata* (8.33%), *C. parapsilosis* (5.8%), *C. guilliermondii* (1.66%) and *C. kefyr* (1.66%). Among 120 *Candida* isolates 38.33% were biofilm positive and 61.66% cases were biofilm negative. Out of total biofilm positive *Candida*, 21.73 % were strong biofilm producers and 78.27% were weak biofilm producers. (Table 1)

Candida sp.	No. (%)	Biofilm negative no. (%)	Biofilm positive no. (%)		
			Strong	Weak	Total
<i>C. albicans</i>	51(42.5)	30 (58.2)	2 (9.5)	19(90.5)	21(41.8)
<i>C. dubliniensis</i>	15 (12.5)	13(86.66)	01(50)	01(50)	02(13.34)
<i>C.tropicalis</i>	19(15.8)	10(52.6)	04 (44.4)	05(55.5)	09(47.4)
<i>C. krusei</i>	14(11.6)	07(50)	01(14.28)	06(85.71)	07(50)
<i>C. glabrata</i>	10(8.3)	07(70)	01(33.33)	02(66.66)	03(30)
<i>C. parapsilosis</i>	07 (5.83)	04(57.14)	01(33.33)	02(66.66)	03(42.85)
<i>C. guilliermondii</i>	02(1.66)	01(50)	-	01(100)	01(50)
<i>C. kefyr</i>	02(1.66)	02(100)	-	-	-
Total	120(100)	74(61.66)	10(21.73)	36(78.27)	46(38.33)

**Table 1: Biofilm formation results of 120 *Candida* isolates by Congo red agar**

**DISCUSSION:** Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation. A biofilm is a community of microorganisms and their extracellular polymers that are attached to a surface.<sup>13</sup> The ability to form biofilms is associated with the pathogenicity and as such should be considered as an important virulence determinant during candidiasis. Biofilms may help fungi in maintaining the role of commensal and pathogen, by evading host immune mechanisms, resisting antifungal treatment, and withstanding the competitive pressure from other organisms. Consequently, biofilm related infections are difficult to treat.<sup>14</sup>

The biofilm production is also associated with high level of antimicrobial resistance of the associated organisms.<sup>15</sup> Out of 120 *Candida* spp. evaluated in this study, 51 (42.5%) were *C. albicans* and 69 (57.5%) were non-*albicans* spp. Among non-*albicans* species *C. tropicalis* 19(15.8%) was the most common isolate. These isolates were tested by in vitro screening test for biofilm production by Congo red agar method. The use of CRA method was simple and reliable to determine whether an isolate has the potential for biofilm production or not and it correlated with study of Jain and Agarwal, 2009.<sup>16</sup> In our study biofilm positivity occurred most frequently in isolates of *C. krusei* followed by *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. albicans*.

This is correlated with Vinitha et al<sup>17</sup> in which a total of 81(73%) out of 111 *Candida* species isolates obtained from the clinical isolates produced biofilm. Only 51% (25 of 49) of *C. albicans* isolates produced biofilm, which was significantly lower than the percentage of all non-*albicans* *Candida* species isolates producing slime (90.32%), 56 of 62;  $P < 0.0001$ ). Strong biofilm production was seen in *C. krusei* and *C. tropicalis*. Weak biofilm production was seen in *C. albicans*. In contrast, Hawser and Douglas<sup>18</sup> reported that isolates of *C. parapsilosis* (Glasgow), *C. pseudotropicalis*, and *C. glabrata* all gave significantly less biofilm growth ( $P < 0.001$ ) than the more pathogenic *C. albicans*.

## ORIGINAL ARTICLE

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The biofilm positivity rates obtained in our study (38.33%) were considered to be an important finding because of the fact that biofilm production is a special feature of *Candida* pathogenicity and its positivity rate matched with study done by Ilknur Dag et al.<sup>19</sup> In which *C. albicans* showed higher percentage of biofilm positivity (39.3%) than non-*albicans* *Candida* strains. (37.79%). In this study, *C. albicans* was the most dominant species isolated from blood, urine, sputum and respiratory specimens followed by *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. kefyr*.

**CONCLUSION:** Our data indicates that the CRA method is a reliable and practical method for determining the biofilm formation of clinical *Candida* isolates. We conclude from our study that CRA method can be recommended as a general screening method for detection of biofilm producing *Candida* in laboratories.

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## ORIGINAL ARTICLE

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