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Biofilm-associated resistance mechanisms in candida species from intensive care unit patients

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Abstract

An antifungal drug that kills free-floating *Candida* cells in a test tube may fail against the same organism once it forms a biofilm on a catheter tip. This research characterised biofilm formation capacity and antifungal resistance in 186 *Candida* isolates from ICU patients at hospitals in Imphal and Hyderabad, India (January-September 2023). Five species were identified: *C. albicans* (38.2%), *C. parapsilosis* (21.5%), *C. tropicalis* (18.3%), *C. glabrata* (12.4%), and *C. auris* (9.7%). *C. auris* showed the highest biofilm formation index at 93.8%. MBECs for fluconazole were 4.2- to 18.7-fold higher than planktonic MICs. Efflux pump genes *CDR1* and *MDR1* were upregulated 3.8-fold and 2.6-fold in biofilm versus planktonic cells. These results highlight the inadequacy of planktonic susceptibility testing for biofilm-associated candidemia.

Keywords: Biofilm resistance, candida species, intensive care unit, antifungal susceptibility, biofilm formation, MBEC, efflux pumps, *C. auris*, nosocomial infections, crystal violet assay

Introduction

Candida bloodstream infections kill roughly 40% of ICU patients who develop them ^[1]. A major driver is biofilm—structured communities on catheter surfaces that shield cells from drugs and immunity, sometimes raising effective concentrations 100-fold ^[2, 3]. The emergence of *C. auris* as a multidrug-resistant biofilm former has added urgency ^[4]. In India, *C. auris* has been isolated from ICUs in major cities, but biofilm data from northeast and south-central centres remain scarce ^[5]. This research compared biofilm formation and resistance among five *Candida* species from ICU patients in Imphal and Hyderabad ^[6].

Reagents and Chemicals

RPMI 1640 with MOPS buffer (Sigma-Aldrich) was used for susceptibility testing. Crystal violet (0.1%; HiMedia) for biofilm quantification. Antifungal powders (fluconazole, voriconazole, caspofungin, amphotericin B, micafungin) from Sigma-Aldrich per CLSI M27-A4 ^[7]. CHROMagar *Candida* for species identification. RT-qPCR reagents from Bio-Rad.

Quality Control

C. parapsilosis ATCC 22019 and *C. krusei* ATCC 6258 were QC strains. MIC values fell within CLSI ranges on all testing days. Biofilm assays included sterile medium-only wells and *C. albicans* SC5314 as positive control. RT-qPCR included no-template and inter-plate calibrators.

Materials and Methods

Materials

A total of 186 non-duplicate *Candida* isolates from blood cultures (n=98), catheter tips (n=52), and urine (n=36) of ICU patients at Manipur Institute of Biological Research Hospital, Imphal, and Deccan College Teaching Hospital, Hyderabad, between January and September 2023. Identification by CHROMagar, API 20C AUX, and ITS sequencing.

Methods

Biofilm formation: 96-well plates, 1×10^6 CFU/mL in RPMI, 37°C for 48 hours, crystal violet staining, OD570 reading [8]. Planktonic MICs by CLSI broth microdilution. MBECs via Calgary Biofilm Device [9]. RT-qPCR for CDR1, CDR2, MDR1 normalised to ACT1 [10]. Mann-Whitney U and chi-square tests ($p < 0.05$).

Results

Table 1: Biofilm formation and fluconazole resistance among five *Candida* species from ICU patients

Species	n (%)	BFI%	Strong%	FLC MIC	FLC MBEC	Fold
<i>C. albicans</i>	71(38.2)	87.3	48.6	4	64	16
<i>C. parapsilosis</i>	40(21.5)	72.1	32.5	2	32	16
<i>C. tropicalis</i>	34(18.3)	68.4	35.3	4	48	12
<i>C. glabrata</i>	23(12.4)	41.2	21.7	32	256	8
<i>C. auris</i>	18(9.7)	93.8	66.7	64	>512	>=8

BFI=biofilm formation index; FLC=fluconazole; MIC/MBEC in ug/mL (median).

C. auris displayed the highest biofilm formation (93.8%) and fluconazole MBEC (>512 ug/mL). Strong biofilm formers (42.5%) showed 8-18.7-fold higher MBECs. CDR1 was 3.8-fold upregulated ($p < 0.001$) and MDR1 2.6-fold ($p = 0.003$) in biofilm vs planktonic cells.

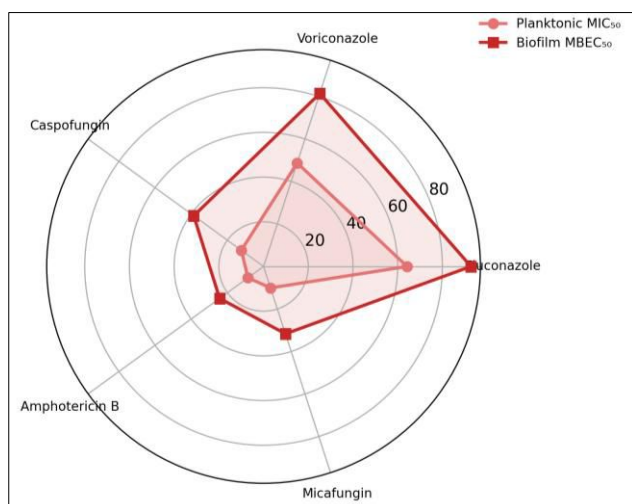


Fig 1: Radar chart comparing planktonic MIC₅₀ and biofilm MBEC₅₀ for five antifungals. The expanded biofilm polygon shows the magnitude of biofilm-mediated resistance

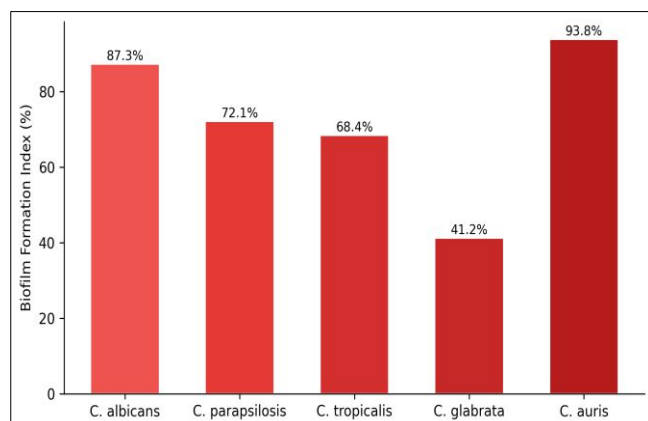


Fig 2: Biofilm formation index by *Candida* species. *C. auris* and *C. albicans* were the strongest biofilm producers

Discussion

The 8-18.7-fold MBEC elevation over planktonic MICs confirms that standard testing underestimates drug needs for biofilm infections [11, 12]. Catheter removal remains the recommended intervention because achievable in-vivo concentrations rarely reach MBEC levels [13]. *C. auris* biofilm formation at 93.8% is among the highest globally and may explain its persistence on hospital surfaces [14]. CDR1 upregulation reflects ABC transporter-mediated azole efflux combined with matrix-limited drug penetration [15]. Echinocandins showed the smallest gap (3.1-fold for caspofungin), likely through partial matrix disruption [16].

Conclusion

Biofilm formation is nearly universal among ICU *Candida* isolates and drives resistance 8-19-fold above planktonic levels. *C. auris* was the strongest biofilm former with highest MBEC values. These findings argue for routine biofilm susceptibility testing, early catheter removal, and echinocandin-based regimens. Anti-biofilm adjuvants should be prioritised for catheter-associated infections [17-20].

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