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Comparative virulence traits of *Candida* spp. in pregnant and non-pregnant women with vulvovaginal candidiasis

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Supplementary material

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Table S1 The cutoff optical density (OD_c) based on negative control replicates used to classify biofilm biomass production by *Candida* spp. isolates

Groups	Mean OD_{nc} of Biological Replicates (R_m)	$R_m - OD_{nc}$	$(R_m - OD_{nc})^2$
G1	0.0515	−0.00283	0.0000080089
G2	0.0545	0.00017	0.0000000289
G3	0.0570	0.00267	0.0000071289
OD_{nc}	**0.05433**		
$\Sigma (R - OD_{nc})^2$	**$\sqrt{[\Sigma (R - OD_{nc})^2 / (n - 1)]}$**	**ODc**	**ODc value**
0.0000151667	**0.002754**	$OD_{nc} + (3 \times SD_{nc})$	0.0626[a]

Sum of squared deviations ($\Sigma (R - OD_{nc})^2$; Standard deviation ($\sqrt{[\Sigma (R - ODnc)^2 / (n - 1)]}$). [a] The calculated ODc value was 0.06259 and was approximated to 0.0626 for subsequent analyses

Table S2 Antifungal susceptibility profiles of *Candida* clinical isolates and reference strains (RS). MIC50 and MIC100 values (μg/mL) of antifungals for *Candida* isolates grouped according to clinical status

G1	Antifungal (μg/mL)						
	MIC50					MIC100	
	VRC	**KTZ**	**FLC**	**ITC**	**MCZ**	**TRB**	**AMB**
*Ca*0007	0.12	2	8	16	0.06	16	2
*Ca*0012	2	0.06	4	0.12	0.06	128	0.25
*Ca*0014	2	0.25	8	0.25	2	64	1
*Ca*0038	0.06	0.06	1	0.06	0.12	8	1
*Cg*0041	0.12	0.25	2	0.5	0.03	128	1
*Ca*0050	0.12	0.06	0.5	0.06	0.06	32	0.25
*Ca*0051	0.25	0.25	0.12	0.25	0.03	16	0.5
*Ca*0060	0.06	0.06	0.06	0.06	0.06	4	0.5
G2	**VRC**	**KTZ**	**FLC**	**ITC**	**MCZ**	**TRB**	**AMB**
*Ca*0021	0.5	0.25	0.5	0.12	0.12	1	16
*Ca*0022	2	8	8	2	1	64	8
*Ca*0025	0.03	0.03	2	0.03	0.03	0.12	8
*Cg*0042	0.25	0.25	4	0.12	0.06	0.25	1
*Ca*0061	0.25	1	2	8	1	256	0.25
*Ca*0062	0.25	0.12	0.25	0.12	1	16	2
*Cg*0063	2	1	8	2	2	256	1
*Ca*0065	2	1	16	1	1	128	1
*Ca*0066	0.5	0.06	0.06	4	0.06	8	0.5
*Ct*0074	0.12	0.03	1	0.5	0.12	128	1
*Ca*0078	0.01	0.25	0.06	0.06	0.06	16	1
*Cp*0092	0.06	0.06	0.25	0.06	0.06	2	0.25
*Cp*0098	0.12	0.06	0.25	0.25	4	1	0.25
G3	**VRC**	**KTZ**	**FLC**	**ITC**	**MCZ**	**TRB**	**AMB**
*Ca*0009	16	0.12	1	0.12	0.03	16	1
*Ca*0059	0.01	0.03	0.12	0.06	0.03	8	0.5
*Ca*0079	0.5	0.06	0.5	0.12	0.12	8	0.12
*Ca*0099	0.03	0.03	0.03	0.03	0.03	0.12	0.12
*Ca*0100	2	0.12	1	0.25	1	256	0.5
*Ca*0105	4	2	8	0.25	1	128	0.12
*Ca*0108	8	0.5	16	2	4	256	1
*Ca*0109	1	0.25	16	2	1	16	1
RS	**VRC**	**KTZ**	**FLC**	**ITC**	**MCZ**	**TRB**	**AMB**
ATCC 64548	0.12	0.03	0.25	0.03	0.03	256	2
ATCC 22019	0.06	0.06	2	0.62	0.06	2	1

AMB, Amphotericin; KTZ, ketoconazole; FLC, Fluconazole; ITC, Itraconazole; MCZ, Miconazole; TRB, Terbinafine; VRC, Voriconazole

Table S3 Tertile-based classification of metabolic activity using absorbance values

Group	Isolates	OD mean	Percentiles	Tertiles
G1	*Ca*0007	2.3765	7/8: 87%	
	*Ca*0012	1.8605	4/8: 50%	
	*Ca*0014	**1.025**	**3/8: 37%**	**T1**
	*Ca*0038	**1.9165**	**5/8: 62%**	**T2**
	*Cg*0041	0.6700	1/8: 12%	
	*Ca*0050	2.2970	6/8: 75%	
	*Ca*0051	3.34	8/8: 100%	
	*Ca*0060	0.8205	2/8: 25%	
G2	*Ca*0021	**1.356**	**4/13: 31%**	**T1**
	*Ca*0022	1.1625	2/13: 15%	
	*Ca*0025	1.649	8/13: 61%	
	*Cg*0042	3.560	12/13: 92%	
	*Ca*0061	1.4815	7/13: 54%	
	*Ca*0062	**1.831**	**9/13: 69%**	**T2**
	*Cg*0063	3,546	13/13: 100%	
	*Ca*0065	3.241	11/13: 85%	
	*Ca*0066	1.873	10/13: 77%	
	*Ct*0074	1.119	1/13: 7%	
	*Ca*0078	1.4065	5/13: 38%	
	*Cp*0092	1.4465	6/13: 46%	
	*Cp*0098	1.3175	3/13: 23%	
G3	*Ca*0009	**1.4685**	**3/8: 37%**	**T1**
	*Ca*0059	1.62533	6/8: 75%	
	*Ca*0079	**1.5875**	**5/8: 62%**	**T2**
	*Ca*0099	1.2105	1/8: 12%	
	*Ca*0100	1.556	4/8: 50%	
	*Ca*0105	1.373	2/8: 25%	
	*Ca*0108	3.5563	7/8: 87%	
	*Ca*0109	3.549	8/8: 100%	

T1 and T2 were defined as the observed values equal to or closest to the tertile cutoffs of 33.3% and 66.7%, respectively

Table S4 Cutoff values used for classification of biofilm metabolic activity in *Candida* isolates from G1–G3

Group	Classification	Cut-off value(s)	Mathematical Criterion
G1	LMA	T1 = 1.025	OD mean ≤ 1.025
	IMA	T1 = 1.025 and T2 = 1.9165	1.025 < OD mean ≤ 1.9165
	HMA	T2 = 1.9165	OD mean > 1.9165
G2	LMA	T1 = 1.356	OD mean ≤ 1.356
	IMA	T1 = 1.356 and T2 = 1.831	1.356 < OD mean ≤ 1.831
	HMA	T2 = 1.831	OD mean > 1.831
G3	LMA	T1 = 1.4685	OD mean ≤ 1.4685
	IMA	T1 = 1.4685 and T2 = 1.5875	1.4685 < OD mean ≤ 1.5875
	HMA	T2 = 1.5875	OD mean > 1.5875

LMA, low metabolic activity; IMA, intermediate metabolic activity; HMA, high metabolic activity. Cut-off values were defined based on the first (T1) and second (T2) tertiles of the absorbance distribution obtained from the MTT assay. Tertiles were calculated separately for each experimental group (G1–G3)