Virulence and adhesive properties of serotypes A and B of *Candida albicans* isolated from paediatric burn patients

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Summary. The virulence and adhesive properties of 50 isolates of *Candida albicans* serotypes A and B collected over 6 years from 48 paediatric burn patients were examined to provide more detailed information about candidal pathogenesis in burn patients and to examine the relevance of the commonly used epithelial cell adhesion assay for determining fungal virulence. The isolates represented a fair distribution of serotypes (29 isolates were serotype A and 21 isolates were serotype B) and a total of 28 serotype-biotype combinations were found; 32% of the serotype-biotype combinations appeared only once, while 44% of the isolates showed similar biotype tests for two of three digits. Adhesion of the isolates to plastic and to buccal epithelial cells (BECs) was examined and compared after growth in a chemically defined medium. There were significant differences in the adhesion of individual isolates to plastic or BECs, but no correlation was found between biotype and adhesiveness. Serotype B isolates were found to be more adhesive to BECs ($p < 0.05$) but not to plastic. There was no apparent correlation between candidal adhesiveness and site of isolation from these patients (autografts, blood, faeces, throat swabs, tracheal aspirates, wounds and intravenous catheters), although isolates from catheters were generally less adhesive to epithelial cells. Virulence in a systemic infection mouse model revealed that there were significant differences in virulence between isolates, but no correlation was found between virulence and the biotype, serotype or site of isolation. Similarly, no correlation was found between virulence and adhesiveness or cell-surface hydrophobicity. These results suggest that, although variations in adhesiveness between isolates of *C. albicans* can be detected in an epithelial cell adhesion assay, such tests may not be useful as reliable predictors of virulence. These data also suggest that candidal pathogenesis is the result of a multifactorial process whereby, in some instances, adhesion may play only a transient role in infection.

Introduction

Infections caused by *Candida albicans* are important causes of morbidity and mortality in burn patients and other immunocompromised individuals.¹ Much has been speculated about the pathogenesis of this organism, and many virulence factors have been proposed as requisites of candidal pathogenicity. The ability of *C. albicans* to adhere to various host tissues and implanted prosthetic devices, for example, is considered to be an important event in colonisation and pathogenesis² and this view is supported by the finding that non-adhesive mutants are less virulent or are inferior in colonisation to their wild-type parental strains.³ In one of the most widely employed techniques for assessing candidal adhesion, exfoliated epithelial cells are used as the test surface.² However,

while the adhesion of *C. albicans* to epithelial cells has been commonly studied² and appears to be a useful test system to assess the virulence of strains and species of candida,^{4–6} no studies have investigated a large number of well characterised isolates to determine the relevance of such tests.² In the studies that have attempted to investigate the virulence of *C. albicans* by determining the ability of isolates to attach to epithelial cells *in vitro*, only 4–6 isolates were examined (half of which have generally been “laboratory strains”) and little or no information has been given with regard to site of isolation, serotype, biotype or virulence in an animal model.⁷ Not surprisingly, these reports are inconsistent in their findings regarding the relationship between adhesiveness of *C. albicans* to exfoliated epithelial cells and virulence.^{2, 8} Studies by King *et al.*⁴, Macura *et al.*⁵ and McCourtie and Douglas,⁶ for instance, showed that there were significant differences in the ability of pathogenic and non-pathogenic *Candida* strains and

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species to adhere to exfoliated epithelial cells *in vitro*. In contrast, Kearns *et al.*⁷ found that differences in adhesiveness between isolates of *C. albicans* obtained from cases of oral thrush and from the mouths of healthy donors were not only small but were inconsistent in relation to their virulence. Therefore, a large gap remains when such adhesion assays are used to define the role of adhesion and certain cell surface properties (e.g., cell surface hydrophobicity) in the overall colonising or pathogenic ability of *C. albicans*.^{2, 8} The purposes of the present study were to examine the adhesiveness and virulence of a large number of clinical isolates of *C. albicans*, to determine if there was a correlation between these properties and serotype or biotype, and to provide more detailed information about *C. albicans* epidemiology and pathogenesis in paediatric burn patients. Also, the relationship of cell-surface hydrophobicity to *C. albicans* adhesion and virulence was examined.

Materials and methods

Candida strains

Fifty isolates of *C. albicans* from various body sites of 48 paediatric burn patients at the Shriners Burn Institute, Cincinnati Unit (Cincinnati, OH, USA) were chosen randomly for study. They were isolated between Jan. 1983 and Dec. 1988 from autografts, blood, faeces, throat swabs, tracheal aspirates, wounds and intravenous catheters. Identification as *C. albicans* was based on the production of germ-tubes after incubation for 2 h in bovine serum and on growth of the yeasts on a pH 1.55 agar.⁹ Isolates were stored at -70°C in skimmed milk-brain heart infusion broth. *C. albicans* strains AK785 and CA30 from the oral cavity of a 1 week-old infant with thrush and the blood of a patient with systemic candidosis, respectively, were used as "standards" for these studies, and served as controls to compare experiments performed on different days.

Growth conditions

For adhesion assays, isolates of *C. albicans* were grown on Sabouraud Maltose Agar (Difco) at $22-24^{\circ}\text{C}$ for 1–4 days. A loopful of cells was then transferred to 100 ml of the medium of Lee *et al.* (designated here LBC)¹⁰ supplemented with 50 mM glucose and the culture was incubated aerobically at 25°C with shaking at 100 rpm.¹¹ For LD50 determinations, yeast cells were grown for 16–18 h on Sabouraud Dextrose Agar (SDA; Difco) at 37°C . Preliminary studies showed that there was no significant difference in virulence of isolates of *C. albicans* grown on SDA or LBC, or at different temperatures. For biotyping, yeasts were streaked on to SDA slants for revival, and then grown on yeast nitrogen base-glucose agar immediately before biotyping.

Cell preparation

After growth, the yeast cells were collected by centrifugation and the pellet was washed twice in either 0.01 M phosphate-buffered saline (PBS)¹¹ at pH 7.2 or buffered KCl (B-KCl)^{12, 13} for adhesion tests with buccal epithelial cells (BECs) or plastic, respectively. The *Candida* cells were suspended to give a final concentration of $2 \times 10^7/\text{ml}$ for adhesion to BECs or $5 \times 10^6/\text{ml}$ for adhesion to plastic. These concentrations were selected because they allowed for maximal adhesion with little or no complication due to co-adhesion.^{11–14}

Mucosal cells

BECs were collected from a healthy female adult by gently rubbing the inside of her cheeks with sterile cotton swabs and suspending the cells in PBS. The donor had no signs or symptoms of oral thrush and had not taken antibiotics for at least 12 months. Cells were washed three times in PBS, resuspended to a concentration of 2×10^5 cells/ml in PBS, as determined by haemocytometer count, and stored at 4°C until required (maximum 2 weeks). Previous studies showed that storage of BECs did not affect the adhesion of *C. albicans*,¹¹ but controls were included throughout the 2-week experimental period to ensure that differences in adhesion were not due to the use of either fresh or stored BECs. Examination by light microscopy confirmed that none of the cell samples contained mucosal cells already colonised by yeasts.

Adhesion assays

Adhesion of *C. albicans* to BECs was assayed as described previously.¹¹ Briefly, 0.2-ml samples of BECs and yeast cells were placed into small test tubes (12×75 mm) and incubated on a rotary shaker (180 rpm) for 1 h at 37°C . After incubation, BECs were collected and washed on polycarbonate filters ($12\text{-}\mu\text{m}$ pore size) (Nucleopore Corporation, Pleasanton, CA, USA). Cells were washed with approximately 100 ml of B-KCl with continual (gentle) agitation. The filters were then stained with Gram's crystal violet, and the number of *C. albicans* adhering to 100 BECs was determined by light microscopy ($\times 430$ magnification). Experiments were repeated three times.

The adhesion of *C. albicans* cells to plastic was studied with polystyrene microtitre trays (Costar, Cambridge, MA, USA) containing 48 11-mm-diameter wells, as described previously.^{12, 13} Briefly, a 0.5-ml sample of a suspension of *C. albicans* cells was placed in each well, the tray was incubated for 1 h at 25°C without shaking, and the wells were then washed three times with 1 ml of assay medium. After incubation of the assay mixture and washing, the trays were inverted and allowed to dry overnight at room temperature. Adherent *C. albicans* were counted by light microscopy, without staining, at $\times 100$; 5–10

1-mm² fields were counted per well, and the assay was performed 4–6 times for each isolate. Only the central area of each well was included in the counting procedure because several non-adherent *C. albicans* cells were deposited at the outer edge of the wells during the drying process. Occasionally, clear patches with few or no yeast cells were observed around or near the centre of the well, and were not included in the areas to be counted. This method provided an efficient and reproducible assay for quantitating the adhesion of *C. albicans* to plastic.^{12, 13}

Phase-partition test for cell surface hydrophobicity

A phase-partition test employing hexadecane as the hydrocarbon phase was used to determine *C. albicans* cell-surface hydrophobicity as described previously.¹⁴ Briefly, 2.5 ml of washed *C. albicans* cells (1×10^8 cells/ml) was mixed vigorously with 0.5 ml of hexadecane and the two liquids were allowed to partition. After separation, the optical density of the aqueous phase was measured at 400 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb, Inc., Rochester, NY, USA). The relative cell-surface hydrophobicity was then determined by measuring the difference in absorbencies of test and control cells, and determining the percentage of cells that had entered the alkane phase.

Serotype testing

C. albicans serotypes were distinguished on the basis of reactions of agglutination (serotype A) or non-agglutination (serotype B) with *Candida*-check antiserum 6 (Iatron, Inc., Tokyo, Japan), as described previously.¹

Biotype testing

The modified method of Odds and Abbott,¹⁵ with commercially available methods of preparing and plating inocula,¹⁶ was used to determine the biotype of each isolate. Briefly, by assessing the growth of each isolate on nine agar plates of various chemical composition, this system determined an isolate's

pH 1.4 and salt tolerance; 5-fluorocytosine, borate and safranin resistance; production of proteinase; and assimilation of urea, sorbose and citrate. Agar plates were inoculated and incubated at 37°C. The nine biochemical tests were grouped in sets containing three tests each. A positive result for each test was assigned a specific numerical value and no growth on a test plate was scored as 0. The summation of these from each set determined a 3-digit biotype for each isolate (table I).

LD50 determinations

The lethality of each isolate was determined in a systemic infection mouse model with 21-day-old female CD-1 mice (Charles Rivers) weighing c. 14 g. After growth on one SDA plate for 16–18 h at 37°C, a lawn of each isolate was suspended in 8 ml of sterile Brain Heart Infusion Broth (BHI; Difco) supplemented with brewers yeast (BHI-BY) 4% v/v final concentration. Serial 10-fold dilutions were made in BHI-BY, and 0.5 ml of *C. albicans* suspension was injected intraperitoneally into each mouse. Each dilution group contained 10 mice and mortality was monitored daily for 2 weeks. The relative virulence of each isolate was compared to the virulence of an experimental control (CA30) to allow direct comparison between isolates tested on different days.

Statistical analyses

To test if there was a statistical difference between serotype A and serotype B of *C. albicans* for distribution in patients, virulence, or a particular adhesive property tested, the one-way analysis of variance test procedure was used. All contrasts were done with $\alpha = 0.05$.

Results

Biotype and serotype distribution

The distribution of serotypes and biotypes among *C. albicans* isolates from various body sites is shown in

Table I. Arrangement of biotyping tests results to give biotype number

Digit 1		Digit 2		Digit 3	
Test	Score for (+) result	Test	Score for (+) result	Test	Score for (+) result
pH 1.4 tolerance	1	Urea assimilation	1	Citrate assimilation	1
Proteinase production	2	Sorbose assimilation	2	Borate resistance	2
5-Fluorocytosine resistance	4	Salt tolerance	4	Safranin resistance	4

The positive results for each test are scored as indicated. All negative results are scored as 0. The sum of the results for the three tests in that column gives the biotype digit.

Table II. Biotypes of *C. albicans* serotypes A and B isolated from paediatric burn patients

Site of isolation	Serotype A		Serotype B	
	Isolate identification	Biotype	Isolate identification	Biotype
Autograft	862391	054	852424	274
	862500	152	872762	056
	880005	350	881110-3	170
	881480	152	881322	456
Blood	840892	076	833325	456
	851446	157		
	870853	072		
Faeces	840276	056	833048	456
	851886	156	843975	376
	860755	152	860710	476
	862703	052	880672	057
Throat swab	851928	056	832847	056
	862042-3	072	853616	456
	870893-2	276	880412-2	656
	871862-3	042		
	872339-3	474		
Tracheal aspirate	840248	056	862400-4	476
	842114	156	870028-3	170
	871124-5	152	880764-3	556
	871764	152	880882-3	472
Wound	840881	076	833005	456
	850383	257	853586	456
	862071-5	270	870097	456
	880435-2	350	872620	477
Intravenous catheter	832890	052	860642	576
	840811	256		
	850189	357		
	851440	157		
	872904-4	072		

table II. The strains represented a fair distribution of serotypes (29 isolates were serotype A and 21 isolates were serotype B) isolated from 48 patients over a 6-year period. Within the 50 isolates examined, a total of 28 serotype-biotype number combinations were found; 32% of the serotype-biotype combinations appeared only once. A total of 44% of all isolates showed similar biotype test results for two of three digits: partial biotypes for the first and second three-set tests were 18%, first and third 14%, and second and third 12%. Of the serotype A isolates, 31% had the partial biotype 15-; 33% of all serotype B isolates had the partial biotype 45-. No general trend was observed for *C. albicans* biotype or serotype and site of isolation. The single exception was for wound isolates for serotype B, of which three of four isolates had a biotype of 456.

Adhesion of C. albicans serotypes and biotypes

To determine whether serotype, biotype or site of isolation had any influence on *C. albicans* adhesion and virulence, all 50 isolates were compared in a battery of experimental models. Since not all strains could be run at the same time for each experiment, two strains of *C. albicans* (AK785 and CA30) that had been well characterised in these systems were used as experimental controls and as a means of comparing

experiments performed on different days. As is summarised in tables III and IV, there was no apparent correlation between the site of isolation and adhesion to plastic or BECs. There were significant differences in adhesiveness between individual isolates and, generally, serotype B was more adhesive than serotype A to plastic. Serotype B (mean adhesion value 30.6 SEM 2.7 *C. albicans*/BEC) was significantly more adhesive to BECs ($p < 0.05$) than serotype A (mean adhesion value 22.4 SEM 1.7 *C. albicans*/BEC), although no one biotype predominated. No analysis could be made of biotypes that appeared singly, but amongst those biotypes that appeared more than once there was no correlation between adhesion to plastic or BECs. The only exception to this was the finding that 83% of biotype 456 isolates showed attachment to BECs that was lower than the median adhesion level, and 88% of isolates with the partial biotype 15- showed attachment to plastic that was lower than the median adhesion level.

Cell-surface hydrophobicity of C. albicans serotypes and biotypes

A comparison was made between the cell-surface hydrophobicity of the isolates and their serotype, biotype and site of isolation. As is summarised in

Table III. Adhesion of *C. albicans* serotypes A and B to plastic

Site of isolation	Serotype A		Serotype B	
	Isolate identification	Mean number of yeast/mm ²	Isolate identification	Mean number of yeast/mm ²
Autograft	862391	10.9	852424	92.9
	862500	41.9	872762	112.6
	880005	2.4	881110-3	11.7
	881480	137.2	881322	81.0
Blood	840892	3.5	833325	125.6
	851446	37.6		
	870853	151.3		
Faeces	840276	62.5	833048	73.3
	851886	4.8	843975	12.8
	860755	42.2	860710	10.8
	862703	5.2	880672	22.6
Throat Swab	851928	29.3	832847	15.8
	862042-3	25.3	853616	17.3
	870893-2	181.6	880412-2	159.5
	871862-3	13.8		
	872339-3	24.5		
Tracheal aspirate	840248	9.6	862400-4	15.4
	842114	9.0	870028-3	272.2
	871124-5	16.3	880764-3	7.0
	871764	1.0	880882-3	182.4
Wound	840881	35.9	833005	140.3
	850383	8.3	853586	159.4
	862071-5	80.9	870097	131.4
	880435-2	68.0	872620	40.0
Intravenous catheter	832890	69.0	860642	91.5
	840811	98.8		
	850189	5.4		
	851440	48.1		
	872904-4	3.7		

table V, there was no significant difference between the cell-surface hydrophobicities of these two serotypes. Furthermore, with the exception of biotype 456 (71% of which had greater cell-surface hydrophobicity than the median level) there was no biotype that showed either an increase or decrease in cell-surface hydrophobicity; however, in general, safranin resistant isolates were more hydrophobic than safranin intolerant ones. A comparison of cell-surface hydrophobicities with adhesion to plastic and BECs revealed that there was no correlation between these properties. Similarly, there was no correlation between cell-surface hydrophobicity and virulence.

Virulence of C. albicans serotypes and biotypes

To determine whether serotype, biotype, site of isolation or adhesiveness had an influence on virulence in a systemic infection mouse model, a comparison was made of the relative virulence of each isolate and these other properties. Table VI summarises the virulence of *C. albicans* serotypes and biotypes in the model. As can be seen from the table, there were significant differences in the virulence of individual strains, but these differences could not be linked to any particular biotype, partial biotype or serotype.

Similarly, there was no significant difference between the virulence of *C. albicans* isolates from various body sites. A comparison of the adhesive properties of individual strains, regardless of serotype, biotype or site of isolation, indicated that there was no apparent correlation between adhesion and virulence in the mouse model used in the present study. Differences in adhesion and virulence were found not to be due to storage of the isolates.

Discussion

There is a large gap in the current knowledge of the pathogenesis of candidal infections. There has been much speculation over the past decade regarding *C. albicans* virulence factors and many determinants of virulence have been proposed.⁹ Similarly, numerous reports have mentioned differences in virulence between serotypes and strains of *C. albicans* or the preponderance of specific strains in certain patient populations.⁹ This has led to the study of isolated factors thought to be of importance in candidal infections as well as to the development of several typing schemes that may serve as predictors of fungal virulence.⁹ In the present study, several factors that

Table IV. Adhesion of *C. albicans* serotypes A and B to buccal epithelial cells (BECs)

Site of isolation	Serotype A				Serotype B			
	Isolate identification	Mean number of <i>C. albicans</i> /BEC	Relative adhesion	Percent of BECs with attached yeasts	Isolate identification	Mean number of <i>C. albicans</i> /BEC	Relative adhesion	Percent of BECs with attached yeasts
Autograft	862391	18.1	68.3	92.0	852424			
	862500	25.8	97.4	100.0	872762	58.0	218.8	100.0
	880005	20.1	75.8	100.0	881110-3	29.7	112.1	94.1
	881480	27.5	103.7	98.0	881322	23.7	89.4	98.0
Blood	840892	24.2	91.3	100.0	833325	16.1	60.7	84.3
	851446	29.6	111.7	100.0				
	870852	37.1	140.0	100.0				
	840276	24.1	90.9	100.0	833048	18.7	70.6	86.0
Faeces	851886	13.5	50.9	90.0	843975	40.6	153.2	100.0
	860755	17.1	64.5	98.0	860710	19.8	74.7	94.1
	862703	37.0	139.6	100.0	880672	33.5	126.4	98.0
	851928	20.3	76.6	100.0	832847	41.5	156.6	98.0
Throat swabs	862042-3	4.9	18.5	92.0	853616	22.5	84.9	100.0
	870893-2	16.6	62.6	98.0	880412-2	57.0	215.1	100.0
	871862-3	13.1	49.4	96.0				
	872339-3	44.5	167.9	100.0				
Tracheal aspirate	840248	29.5	111.3	100.0	862400-4	19.0	71.7	98.0
	842114	23.8	89.8	96.1	870028-3	27.3	103.0	100.0
	871124-5	19.9	75.1	96.0	880764-3	17.9	67.5	72.0
	871764	22.9	86.4	100.0	880882-3	31.4	118.5	100.0
Wound	840881	28.5	107.5	100.0	833005	34.3	129.4	100.0
	850383	9.2	34.7	88.2	853586	24.9	93.9	98.0
	862071-5	28.2	106.4	100.0	870097	20.4	77.0	96.0
	880435-2	31.3	118.1	98.0	872620	30.9	116.6	100.0
Intravenous catheter	832890	12.4	46.8	100.0	860642	44.3	167.2	100.0
	840811	15.8	59.6	88.0				
	850189	9.2	34.3	80.4				
	851440	19.6	74.0	100.0				
	872904-4	25.3	95.5	100.0				

$$\text{Relative adhesion} = \frac{\text{Mean number of yeast cells (test isolate)/epithelial cell}}{\text{Mean number of yeast cells (AK 785 control)/epithelial cell}} \times 100.$$

Table V. Cell-surface hydrophobicity (CSH) of *C. albicans* serotype A and B

Site of isolation	Serotype A		Serotype B	
	Isolate identification	Percent of cells entering the alkane phase	Isolate identification	Percent of cells entering the alkane phase
Autograft	862391	6.2	852424	0
	862500	13.6	872762	0
	880005	9.1	881110-3	0
	881480	15.0	881322	13.6
Blood	840892	18.2	833325	23.6
	851446	9.1		
	870853	8.3		
Faeces	840276	22.7	833048	4.5
	851886	0	843975	10.8
	860755	11.9	860710	19.2
	862703	0.9	880672	20.8
Throat swabs	851928	25.0	832847	8.3
	862042-3	0	853616	13.0
	870893-2	8.3	880412-2	22.8
	871862-3	5.0		
Tracheal aspirate	872339-3	13.7		
	840248	16.7	862400-4	27.3
	842114	0	870028-3	0.8
	871124-5	11.4	880764-3	6.5
Wound	871764	0	880882-3	17.5
	840881	20.4	833005	18.2
	850383	16.8	853586	18.1
	862071-5	27.3	870097	2.4
Intravenous catheter	880435-2	9.1	872620	6.8
	832890	0	860642	9.5
	840811	15.5		
	850189	6.8		
	851440	33.3		
	872904-4	18.7		

might influence virulence were examined in isolates from one pathogenic setting—paediatric burn patients.

The most widely studied virulence factor for *C. albicans* is attachment to epithelial cells.¹⁷ Although recent studies by Calderone and co-workers have shown through the use of non-adhesive mutants that adhesion to host tissues plays an important role in *C. albicans* virulence,³ it has not been determined whether a transient or permanent adhesion is required for pathogenesis. Thus, while adhesion may be required to initiate infection, these initial adhesive interactions may later give way to some other means of tissue localisation (e.g., entrapment in liver sinusoids or fibrin-platelet clots), in which case some other virulence mechanism would be likely to be of more importance.² The impression given by some reports that adhesion is the most important virulence factor in candidal infections, or that simple tests such as adhesion to exfoliated epithelial cells can be used to assess fungal virulence, remain to be proved.

The results of the present study reaffirm that adhesion alone does not account for *C. albicans* virulence and that this property cannot be relied upon as a predictor of virulence for this organism. The present data also confirm the findings of Hasenclever

and Mitchell¹⁸ that differences in virulence could not be distinguished on the basis of serotype. Furthermore, these data are in agreement with the suggestion that current biotyping schemes for *C. albicans* cannot identify strains with high or low virulence attributes.⁹ It was also of particular interest to note that there was no apparent difference in adhesive or virulence abilities of the strains related to site of isolation.

Perhaps the most striking findings in this study was the increased ability of *C. albicans* serotype B to adhere to BECs. This was particularly striking because there was no significant difference in cell-surface hydrophobicity, adhesion to plastic or virulence in mice. Analysis of mannans isolated from serotypes A and B of *C. albicans* has revealed differences between the mannans of these serotypes. These differences appear to be due to extent of branching, the length of the side chain (serotype A is thought to have longer side chains), and the proportion and position of α -1,2-linkages in the side chains.² Whether these differences may account for the difference in adhesion to BECs is not known.²

The finding that there was no correlation between cell-surface hydrophobicity and adhesion to either BECs or plastic is consistent with recent findings.¹¹⁻¹⁴ For instance, white and opaque phenotypes of

Table VI. Virulence of *C. albicans* serotypes A and B in a systemic infection mouse model

Site of isolation	Serotype A				Serotype B			
	Isolate identification	LD50	95% confidence limits	Relative virulence (%)	Isolate identification	LD50	95% confidence limits	Relative virulence (%)
Autograft	862391	8.8 × 10 ⁶	(3.4 × 10 ⁶ –2.2 × 10 ⁷)	0.1	852424	> 3.6 × 10 ⁸	—	0.2
	862500	8.6 × 10 ⁶	(2.9 × 10 ⁶ –2.5 × 10 ⁷)	7.2	872762	2.1 × 10 ⁶	(2.0 × 10 ⁵ –6.4 × 10 ⁶)	3.5
	880005	4.4 × 10 ⁶	(1.6 × 10 ⁶ –1.1 × 10 ⁷)	14.1	881110–3	3.0 × 10 ⁵	(4.8 × 10 ⁴ –1.2 × 10 ⁶)	3.3
	881480	2.7 × 10 ⁸	(8.0 × 10 ⁷ –1.1 × 10 ⁹)	0.3	881322	2.0 × 10 ⁶	(6.8 × 10 ⁵ –5.9 × 10 ⁶)	31.0
	840892	4.9 × 10 ⁸	(9.0 × 10 ⁷ –4.5 × 10 ⁹)	0.1	833325	2.5 × 10 ⁷	(9.6 × 10 ⁶ –6.9 × 10 ⁷)	1.5
Blood	851446	1.0 × 10 ⁷	(4.0 × 10 ⁶ –3.0 × 10 ⁷)	0.7				
	870853	7.5 × 10 ⁶	(2.8 × 10 ⁶ –1.9 × 10 ⁷)	8.3				
	840276	2.4 × 10 ⁶	(9.2 × 10 ⁵ –6.1 × 10 ⁶)	0.4	833048	4.7 × 10 ⁷	(1.7 × 10 ⁷ –1.2 × 10 ⁸)	1.3
Faeces	851886	8.4 × 10 ⁷	(3.2 × 10 ⁷ –2.3 × 10 ⁸)	0.01	843975	5.7 × 10 ⁵	(1.6 × 10 ⁵ –1.7 × 10 ⁶)	108.8
	860755	2.7 × 10 ⁶	(6.4 × 10 ⁵ –1.0 × 10 ⁷)	0.4	860710	> 3.7 × 10 ⁸	—	0.8
	862703	1.1 × 10 ⁶	(3.4 × 10 ⁵ –3.1 × 10 ⁶)	56.4	880672	1.1 × 10 ⁸	(3.6 × 10 ⁷ –3.8 × 10 ⁸)	0.8
	851928	4.5 × 10 ⁷	(1.7 × 10 ⁷ –1.2 × 10 ⁸)	2.0	832847	2.2 × 10 ⁶	(5.0 × 10 ⁵ –9.5 × 10 ⁶)	10.9
	862042–3	1.8 × 10 ⁶	(5.9 × 10 ⁵ –5.6 × 10 ⁶)	4.1	853616	2.6 × 10 ⁵	(5.6 × 10 ⁴ –9.7 × 10 ⁵)	28.1
Throat swabs	870893–2	1.7 × 10 ⁷	(3.5 × 10 ⁶ –8.0 × 10 ⁷)	1.4	880412–2	6.7 × 10 ⁷	(2.5 × 10 ⁷ –1.8 × 10 ⁸)	0.01
	871862–3	3.2 × 10 ⁵	(1.1 × 10 ⁵ –9.0 × 10 ⁵)	22.8				
	872339–3	1.3 × 10 ⁷	(5.1 × 10 ⁶ –3.5 × 10 ⁷)	4.8				
	840248	7.2 × 10 ⁶	(2.1 × 10 ⁶ –2.4 × 10 ⁷)	8.6	862400–4	5.0 × 10 ⁷	(1.9 × 10 ⁷ –1.4 × 10 ⁸)	1.8
	842114	1.5 × 10 ⁷	(5.0 × 10 ⁶ –4.9 × 10 ⁷)	0.5	870028–3	6.7 × 10 ⁵	(1.6 × 10 ⁵ –2.4 × 10 ⁶)	92.5
Tracheal aspirate	871124–5	1.3 × 10 ⁵	(3.9 × 10 ⁴ –4.1 × 10 ⁵)	56.2	880764–3	9.7 × 10 ⁷	(3.7 × 10 ⁷ –3.8 × 10 ⁸)	0.9
	871764	6.1 × 10 ⁷	(2.3 × 10 ⁷ –1.7 × 10 ⁸)	1.0	880882–3	4.2 × 10 ⁷	(1.6 × 10 ⁷ –1.1 × 10 ⁸)	0.02
	840881	4.8 × 10 ⁶	(9.7 × 10 ⁵ –1.8 × 10 ⁷)	5.0	833005	2.6 × 10 ⁶	(9.6 × 10 ⁵ –7.0 × 10 ⁶)	2.9
Wound	850383	1.8 × 10 ⁴	(1.1 × 10 ⁴ –2.0 × 10 ⁵)	1333.3	853586	3.5 × 10 ⁶	(1.3 × 10 ⁶ –9.3 × 10 ⁶)	17.7
	862071–5	2.7 × 10 ⁷	(8.7 × 10 ⁶ –1.0 × 10 ⁸)	2.3	870097	2.4 × 10 ⁴	(2.4 × 10 ³ –1.5 × 10 ⁵)	2583.3
	880435–2	1.6 × 10 ⁷	(5.5 × 10 ⁶ –5.1 × 10 ⁷)	5.6	872620	2.5 × 10 ⁷	(9.4 × 10 ⁶ –6.7 × 10 ⁷)	3.6
IV catheter	832890	2.9 × 10 ⁶	(6.2 × 10 ⁵ –1.2 × 10 ⁷)	8.2	860642	4.4 × 10 ⁶	(1.6 × 10 ⁶ –1.1 × 10 ⁷)	20.5
	840811	8.5 × 10 ⁷	—	0.1				
	850189	4.7 × 10 ⁷	(1.2 × 10 ⁷ –1.7 × 10 ⁸)	1.3				
	851440	4.9 × 10 ⁶	(1.7 × 10 ⁶ –1.4 × 10 ⁷)	12.6				
	872904–4	4.0 × 10 ⁷	(1.5 × 10 ⁷ –1.0 × 10 ⁸)	2.2				

Relative virulence: (LD50 of *C. albicans* CA30/LD50 of test isolate) × 100.

C. albicans were found to differ significantly in their cell-surface hydrophobicity but this difference did not account for the difference in adhesive properties between these two isolates.^{12,13} Similarly, other studies that have examined the possible role of cell surface hydrophobicity in adhesion to BECs and intestinal mucosa, suggest that this property plays only

a minor role in these processes.¹⁹ The present study, together with the latter studies, seem to cast doubt on the suggestion that cell-surface hydrophobicity is the principle mechanism of adhesion to plastic and suggests that this property should be studied in greater detail to determine its precise role in the adhesion of *C. albicans* to biological and non-biological surfaces.

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