Silicone colonization by non-*Candida albicans* *Candida* species in the presence of urine

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Urinary tract infections (UTIs) are the most common nosocomial infections and 80 % are related to the use of urinary catheters. Furthermore, *Candida* species are responsible for around 15 % of UTIs and an increasing involvement of non-*Candida albicans Candida* (NCAC) species (e.g. *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis*) has been recognized. Given the fact that silicone is frequently used in the manufacture of urinary catheters, the aim of this work was to compare both the adhesion and biofilm formation on silicone of different urinary clinical isolates of NCAC species (i.e. *C. glabrata*, *C. tropicalis* and *C. parapsilosis*) in the presence of urine. Several clinical isolates of NCAC species recovered from patients with UTIs, together with reference strains of each species, were examined. Adhesion and biofilm formation were performed in artificial urine and the biofilm biomass was assessed by crystal violet staining. Hydrophobicity and surface charge of cells was determined by measuring contact angles and zeta potential, respectively. The number of viable cells in biofilms was determined by enumeration of c.f.u. after appropriate culture. The biofilm structure was also examined by confocal laser scanning microscopy (CLSM). The results showed that all isolates adhered to silicone in a species- and strain-dependent manner with *C. parapsilosis* showing the lowest and *C. glabrata* the highest levels of adhesion. However, these differences in adhesion abilities cannot be correlated with surface properties since all strains examined were hydrophilic and exhibited a similar zeta potential. Despite a higher number of cultivable cells being recovered after 72 h of incubation, stronger biofilm formation was not observed and CLSM showed an absence of extracellular polymeric material for all isolates examined. In summary, this work demonstrated that all tested NCAC species were able to adhere to and survive on silicone in the presence of urine. Furthermore, *C. glabrata* strains presented higher colonization abilities than *C. tropicalis* and *C. parapsilosis* strains, a fact that might explain the larger role of *C. glabrata* colonization and disseminated infections in hospitalized and catheterized patients.

INTRODUCTION

Nosocomial infections constitute a serious public health problem, as they are a major cause of morbidity and mortality, and cause an increased time of hospitalization with associated enhanced healthcare costs (Tamura *et al.*, 2003). Urinary tract infection (UTI) is one of the most common types of nosocomial infections (Schaberg *et al.*, 1991; Tamura *et al.*, 2003) and more than 80 % of nosocomial UTIs are related to the use of medical devices such as urinary catheters (Febré *et al.*, 1999; Harris *et al.*, 1999; Douglas, 2002; Chow *et al.*, 2008). Furthermore, *Candida* species are responsible for around 80 % of fungal infections in the hospital environment and 10–15 % of UTIs are caused by these micro-organisms (Amer *et al.*, 2004). Although the majority of infections are caused by *Candida albicans* (Richards *et al.*, 1999; Sobel *et al.*, 2000), non-*C. albicans Candida* (NCAC) species including *Candida glabrata* (Harris *et al.*, 1999; Manzano-Gayosso *et al.*, 2008), *Candida tropicalis* (Kauffman *et al.*, 2000) and *Candida parapsilosis* (Ruan *et al.*, 2008) are emerging as important nosocomial pathogens with a predilection for the urinary tract.

Candida species are commensal micro-organisms that become pathogenic when the defence mechanisms of the host are weakened, and these organisms then have the ability to cause a variety of superficial and systemic

Abbreviations: AU, artificial urine; CLSM, confocal laser scanning microscopy; NCAC, non-*Candida albicans Candida*; UTI, urinary tract infection.

infections (Kojic & Darouiche, 2004). The primary event in *Candida* infection is the adherence of the micro-organism to a host and/or medical device surface, often leading to the formation of biofilms (Crump & Collignon, 2000; Chandra *et al.*, 2001a). Once established, *Candida* biofilms serve as a persistent reservoir of infection and, in addition, offer great tolerance to antifungal agents (Chandra *et al.*, 2001b; Ramage *et al.*, 2001; Samaranayake *et al.*, 2005).

Predisposition to the development of fungal infections is mediated by multiple factors (Douglas, 2003) and it is now recognized that implanted devices have a profound clinical impact due to *Candida*-associated biofilms (Douglas, 2002). Thus the main aim of this work was to compare both the adhesion and biofilm formation ability of different urinary clinical isolates of NCAC species (i.e. *C. glabrata*, *C. tropicalis* and *C. parapsilosis*) onto silicone in the presence of urine.

METHODS

Organisms. A total of six urinary isolates of *C. parapsilosis* (534638 and 553877), *C. tropicalis* (519468 and 544123) and *C. glabrata* (562123 and 513100) were used in the course of this study. Three reference strains, namely *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750 and *C. glabrata* ATCC 2001, were also examined. Strains isolated from UTIs were gifts from the collection of the Hospital of São Marcos (Braga, Portugal). The identity of all isolates was confirmed using CHROMagar Candida and by PCR sequencing using specific primers (ITS1 and ITS4) targeting the 5.8S rRNA subunit gene (Williams *et al.*, 1995). Genomic DNA was extracted according to previously described procedures (Scherer & Stevens, 1987). PCR products were sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing kit (Perkin Elmer, Applied Biosystems).

Medium and growth conditions. All *Candida* isolates were subcultured on Sabouraud dextrose agar (SDA; Merck) for 48 h at 37 °C, followed by culture in Sabouraud dextrose broth (Merck) for 18 h at 37 °C in an orbital shaker (120 r.p.m.). Cells were then harvested by centrifugation at 3000 ***g*** for 10 min at 4 °C and washed twice with PBS (pH 7, 0.1 M). The cell pellets were finally resuspended in artificial urine (AU) and cell concentration was adjusted using an improved Neubauer haemocytometer to 1×10^7 cells ml^{-1}. AU (pH 5.8) was prepared with slight modification to that previously described (Shin *et al.*, 2002; Jain *et al.*, 2007). The composition of the AU used in this study was: $CaCl_2$, 0.65 g l^{-1}; $MgCl_2$, 0.65 g l^{-1}; NaCl, 4.6 g l^{-1}; Na_2SO_4, 2.3 g l^{-1}; $Na_3C_3H_5O(CO_2)_3$, 0.65 g l^{-1}; $Na_2C_2O_4$, 0.02 g l^{-1}; KH_2PO_4, 2.8 g l^{-1}; KCl, 1.6 g l^{-1}; NH_4Cl, 1.0 g l^{-1}; urea, 25.0 g l^{-1}; creatinine, 1.1 g l^{-1}; and glucose, 0.3 %.

Silicone. Coupons (1×1 cm^2) were cut from a 1 mm layer of silicone (Neves & Neves). This material was chosen due to its similarity to the material used in indwelling devices, including urinary catheters. All coupons were cleaned by immersion in ultrapure water, followed by immersion in 50 % (v/v) ethanol for 4 h. After rinsing with ultrapure water and air-drying, the coupons were autoclaved for 15 min at 121 °C.

Candida surface properties

Contact angle measurement. Hydrophobicity was evaluated through contact angle measurements and using the approach of van Oss *et al.* (1987, 1989). The measurements were made on *Candida* cell lawns deposited on membrane filters, after 2 h of equilibrium in AU pH 5.8, prepared according to Busscher *et al.* (1984). For this, suspensions of 5 ml of 10^8 cells ml^{-1} were filtered and deposited over solidified agar plates (2 % agar and 10 % glycerol) and dried at 37 °C for 3–4 h to standardize the humidity level. Contact angles were measured by the sessile drop technique on the cell lawns using a contact angle measurement apparatus (model OCA 15 PLUS; DATAPHYSICS). The measurements were performed at room temperature, using three different liquids: water, formamide and 1-bromonaphthalene. Each assay was performed in triplicate and at least 20 contact angles per sample were measured.

Zeta potential measurement. The electric charge of the *Candida* strains under study was evaluated by zeta potential determination after 2 h of equilibrium in AU pH 5.8 (1×10^7 cells ml^{-1}). Measurements were performed with a microelectrophoresis cell (DLS Nanosizer; Malvern). The applied voltage was 200 V, and each mean value consisted of 25 recordings.

Adhesion assays. For the adhesion assays, silicone coupons were placed into 12-well microtitre plates (Orange Scientific) and 1 ml of the standardized cell suspension (1×10^7 cells ml^{-1} in AU) was added. For controls, silicone coupons were similarly processed but in the absence of *Candida* cells. The microtitre plates were incubated for 2 h at 37 °C in an orbital shaker (120 r.p.m.). Following incubation, silicone coupons were placed into new 12-well culture plates and washed once with ultrapure water to remove non-adhered cells. The cells were then fixed with 1 ml 100 % (v/v) methanol, which was removed after 15 min of contact. The silicone coupons were then allowed to air dry at room temperature, and 1 ml 1 % (v/v) crystal violet (CV) was added and incubated for 5 min to stain the adhered cells. The silicone coupons were then gently washed twice with ultrapure water to remove the excess of CV and were allowed to dry at room temperature.

Coupons were visualized under a light microscope (Olympus BX51) coupled to a DP71 digital camera (Olympus) for acquisition of images (original magnification $\times 400$). At this magnification, each captured image was equivalent to 1.7×2.2 cm^2. For each surface analysed, at least 20 images were obtained across the entire silicone surface. The number of adhered cells per image was determined by image analysis using automated enumeration software (Sigma Scan Pro; Systat Software). The results were then expressed as the number of cells per unit area of silicone (number cells cm^{-2}). All experiments were performed in triplicate and in three to five independent assays.

Biofilm formation assays. For biofilm formation assays, silicone coupons were placed in 12-well microtitre plates (Orange Scientific) and 1 ml standardized cell suspension (1×10^7 cells ml^{-1} in AU) was added. The microtitre plates were incubated for 4, 8, 12, 24, 48 and 72 h at 37 °C in an orbital shaker (120 r.p.m.). Every 24 h, 500 µl AU was removed and an equal volume of fresh AU was added. For controls, silicone coupons were similarly processed but in the absence of *Candida*. Biofilm formation was assessed by total biomass quantification using CV staining (Stepanovic *et al.*, 2000; Silva *et al.*, 2009). Thus, after the defined times of incubation, the medium was aspirated and non-adherent cells were removed by washing the silicone coupons with sterile water. The biofilm was then fixed with 1 ml 100 % (v/v) methanol, which was removed after 15 min of contact. The silicone coupons were allowed to dry at room temperature, and 1 ml CV (1 %, v/v) was added to each well and incubated for 5 min. The silicone coupons were then gently washed with sterile water and 1 ml acetic acid (33 %, v/v) was added to release the CV from cells. The A_{570} of the obtained solution was read in triplicate in a microtitre plate reader (Bio-Tek Synergy HT; Izasa). The final absorbance was standardized according to the area of

silicone (absorbance cm^{-2}). Experiments were performed in triplicate and repeated in three to five independent assays.

***Candida* viability assays.** The number of cultivable cells was determined by c.f.u. enumeration. Briefly, after 4 and 72 h of biofilm formation (as described previously), silicone coupons were washed once in PBS (0.1 M, pH 7) to remove loosely attached cells. Coupons of silicone were then immersed in 3 ml PBS (0.1 M, pH 7) in new 6-well tissue culture plates and sonicated (Ultrasonic Processor; Cole-Parmer) for 45 s at 30 W. Complete removal of adhered cells was confirmed by CV staining (as described previously). The obtained suspensions were vortexed vigorously for 5 min and then serial decimal dilutions (in PBS) were subcultured onto SDA and incubated for 24 h at 37 °C for c.f.u. enumeration (log c.f.u. cm^{-2}). Experiments were repeated on three occasions with individual samples evaluated in triplicate.

Confocal laser scanning microscopy (CLSM). After 72 h of biofilm formation, silicone coupons were washed once with PBS (0.1 M, pH 7), incubated for 40 min at 37 °C in 1 ml PBS containing the fluorescent stains FUN 1 (10 μM; Molecular Probes-Invitrogen) and concanavalin A Alexa Fluor 488 conjugate (25 μg ml^{-1} in PBS; Molecular Probes-Invitrogen) and observed by CLSM.

FUN 1 (excitation wavelength=543 nm and emission=560 nm) is converted to orange–red cylindrical intravacuolar structures by metabolically active cells, while concanavalin A (excitation wavelength=488 nm and emission=505 nm) binds to glucose and mannose residues with the emission of green fluorescence. Stained silicone coupons were observed with a Leica TCS SP2 AOBS spectral confocal microscope.

Statistical analysis. Results were compared using one-way analysis of variance (ANOVA) by applying Levene's test of homogeneity of variance and the Tukey multiple-comparisons test, using SPSS software. All tests were performed with a confidence level of 95 %.

RESULTS

Hydrophobicity and zeta potential of NCAC species

Candida cell surface physico-chemical characteristics are presented in Table 1. The hydrophobicity of the yeast cell surfaces (ΔG_{sws}) expresses the free energy of interaction between two identical surfaces (s) immersed in water (w), as proposed by van Oss (1995). Water contact angles were used as a qualitative indication of cell surface hydrophobicity, with values below 65° indicating a hydrophilic surface (Vogler, 1998).

Similar water contact angle values were obtained for all NCAC strains, ranging from 17.3° ± 1.2 (*C. parapsilosis* 553877) to 24.0° ± 1.3 (*C. tropicalis* 544123), indicating that the different strains were of similar cell surface hydrophobicity ($P>0.05$). The low (<50°) contact angle values indicated hydrophilic surfaces. Regarding surface tension components, all strains showed higher values of the electron donor parameter (γ^{-}) compared to the electron acceptor parameter (γ^{+}). *C. tropicalis* 544123 showed, again, the highest value of electron acceptance while *C. glabrata* ATCC 2001 revealed the lowest value of the electron donor parameter.

Table 1 summarizes the zeta potential values obtained for different NCAC strains when immersed in AU pH 5.8. All strains had a negative zeta potential value, ranging from −4.2 ± 0.6 mV to −1.4 ± 0.0 mV. Furthermore, *C. glabrata* strains presented the most negative values, which were statistically lower than those of the *C. parapsilosis* and *C. tropicalis* strains ($P<0.05$).

Adhesion of NCAC species to silicone

Fig. 1 presents the results of the extent of adhesion of NCAC species (i.e. *C. glabrata*, *C. tropicalis* and *C. parapsilosis*) to silicone in the presence of AU. It was evident that all NCAC strains adhered to silicone, although differences were observed according to species and strains. Strain variation was particularly evident for *C. parapsilosis*, with *C. parapsilosis* 534638 displaying the highest number of cells adhered to silicone ($2.2\times10^{6}\pm7.5\times10^{5}$ cells cm^{-2}). This was significantly higher than for *C. parapsilosis* 553877

Table 1. Water contact angle (θ), values of the polar components of surface tension (γ^{+}, γ^{-}), degree of hydrophobicity (ΔG_{sws}) and zeta potential (ζ) of NCAC strains assayed

SD, Standard deviation.

Species	Strain	θ (°) ± SD	γ^{+} (mJ m^{-2})	γ^{-} (mJ m^{-2})	ΔG_{sws} (mJ m^{-2})	ζ (mV) ± SD
C. parapsilosis	534638	19.4 ± 2.7	4.4	51.1	24.4	−2.2 ± 0.0
	553877	17.3 ± 1.2	3.4	51.8	26.3	−1.4 ± 0.0
	ATCC 22019	17.7 ± 2.5	3.6	51.6	25.8	−2.7 ± 0.5
C. tropicalis	519468	20.7 ± 1.3	3.7	49.0	23.2	−2.6 ± 0.1
	544123	24.0 ± 1.3	8.4*	47.2*	15.5*	−2.6 ± 0.6
	ATCC 750	20.5 ± 2.3	4.5	49.8	23.0	−3.8 ± 0.7
C. glabrata	562123	17.4 ± 2.4	3.4	50.6	25.1	−4.1 ± 0.6†
	513100	19.2 ± 2.4	4.9	51.3	23.7	−4.1 ± 0.9†
	ATCC 2001	19.6 ± 2.2	3.1	53.0	28.4	−4.2 ± 0.6†

*Statistically different from the other strains ($P<0.05$).

†Strains that are statistically similar ($P>0.05$) but different from ($P<0.05$) those of other species.

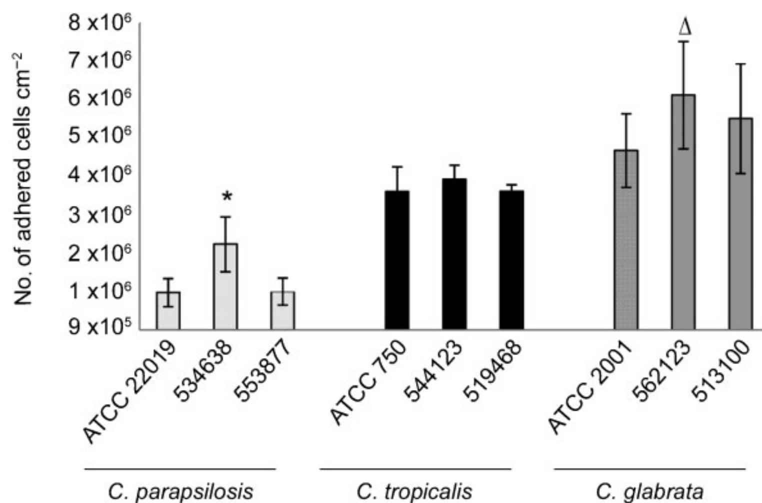

Fig. 1. Number of cells of NCAC species per unit area adhered to silicone after 2 h in the presence of AU. Error bars represent standard deviation. *Statistically different from the other strains of the same species ($P<0.05$); Δstatistically different from all strains of other species ($P<0.05$).

(P=0.024) and the reference strain *C. parapsilosis* ATCC 22019 (P=0.023). Compared with *C. tropicalis* and *C. glabrata*, *C. parapsilosis* adhered to the silicone coupons at a significantly lower ($P<0.05$) level. Strains of *C. glabrata* adhered at equivalent levels to silicone ($P>0.05$), with the highest number of adhered cells (6.2×10^6 cells cm^{-2}) evident for *C. glabrata* 562123. *C. tropicalis* strains equally showed similar adhesion levels with little inter-strain difference ($P>0.05$).

Biofilm formation of NCAC species on silicone

Biofilm quantification was performed through total biomass quantification by CV staining. Fig. 2 shows the analysis of biofilm development (4, 8, 12, 24, 48 and 72 h of incubation) for *C. parapsilosis*, *C. tropicalis* and *C. glabrata* strains on silicone in the presence of AU. It was evident that biofilms of *C. glabrata* had a higher total biomass than those of *C. parapsilosis* and *C. tropicalis* at all measured time points ($P<0.05$). *C. tropicalis* and *C. parapsilosis* strains produced biofilm biomass of similar levels ($P>0.05$), whilst *C. glabrata* strains exhibited a more heterogeneous behaviour under the test conditions. No statistical differences were observed between biomass values obtained at 4 and 72 h in the case of *C. tropicalis* and *C. parapsilosis* strains ($P>0.05$). In contrast, biomass values for *C. glabrata* 513100 and ATCC 2001 strains decreased significantly after 48 h of incubation ($P<0.05$). Silicone coupons incubated for 72 h were also examined by fluorescence CLSM (Fig. 3). The intense green fluorescence, resulting from concanavalin A binding to polysaccharides, outlines the live and dead cell wall of yeasts, while the red/orange colour, due to FUN 1 staining, identifies active cells. Thus confocal images showed an absence of extracellular polymeric material (green coloration) at the last period of incubation (cells separated by regions lacking fluorescence), though this is not surprising, since the concentration of polymers within the matrix is typically low. The observed area of red/orange fluorescence confirmed the presence of higher amounts of metabolically active cells after 72 h adhesion on silicone in the presence of urine. Furthermore, *C. parapsilosis* and *C. tropicalis* strains presented filamentous morphologies, which were not evident with *C. glabrata* strains.

DISCUSSION

UTI is the most common type of nosocomial infection and is frequently associated with the use of indwelling urinary catheters (Febré *et al.*, 1999). *Candida* species are responsible for around 15 % of UTIs (Amer *et al.*, 2004) and there is an increasingly recognized involvement of NCAC species (Harris *et al.*, 1999; Kauffman *et al.*, 2000; Krcmery & Barnes, 2002; Ruan *et al.*, 2008). Silicone rubber is the biomaterial frequently used to manufacture urinary catheters, and given the involvement of NCAC in UTIs, this present study aimed to assess the ability of NCAC species to colonize silicone in the presence of AU.

All NCAC species (i.e. *C. glabrata*, *C. parapsilosis* and *C. tropicalis*) were able to adhere to silicone (Fig. 1), which is in agreement with other studies examining *Candida* species adherence to silicone rubber (Busscher *et al.*, 1997; Tamura *et al.*, 2003). However, it is important to note that *C. glabrata* strains adhered to a higher extent than *C. parapsilosis* and *C. tropicalis*. In fact, *C. glabrata* strains have frequently been isolated from patients with candiduria (Harris *et al.*, 1999; Manzano-Gayosso *et al.*, 2008) and are recognized as potentially pathogenic in hospitalized and catheterized patients (Fidel *et al.*, 1999). As previously reported (Trofa *et al.*, 2008), *C. parapsilosis* is not a frequent aetiological agent of UTIs. This was also evident in a recent epidemiological study of 100 candiduria cases (between 1999 and 2004) in a paediatric hospital in Brazil. In this study, *C. parapsilosis* was only isolated on four occasions compared with 56 for *C. albicans*, 20 for *C. tropicalis* and 11 for *C. glabrata* (da Silva *et al.*, 2007). Furthermore, despite the apparently reduced ability to

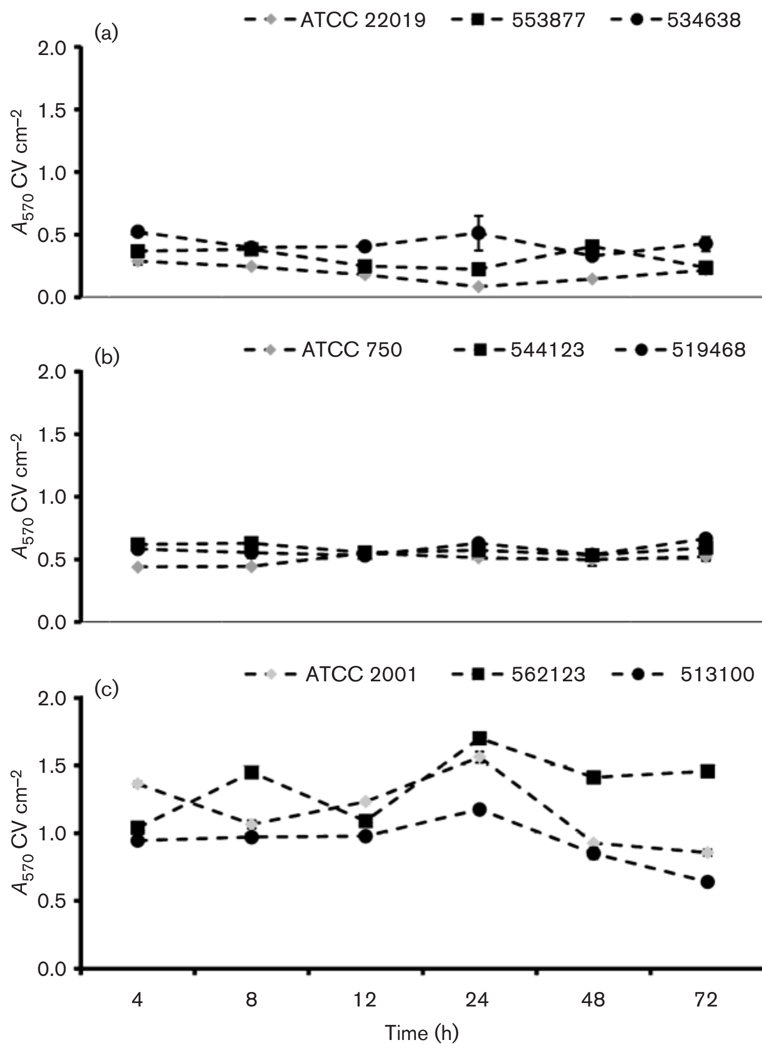

Fig. 2. A_{570} of CV solutions obtained from biofilms of NCAC species formed on silicone in the presence of AU. (a) *C. parapsilosis*; (b) *C. tropicalis*; and (c) *C. glabrata*. Error bars represent standard deviation.

adhere to silicone, *C. parapsilosis* strains exhibited a higher degree of intra-species heterogeneity compared with the two other species examined (Fig. 1). This intra-species variation by *C. parapsilosis* in its adherence to different biomaterials has been reported previously (Cassone *et al.*, 1995; Panagoda *et al.*, 2001; Trofa *et al.*, 2008). Such findings undoubtedly reflect inherent physiological differences between species and strains and could have significance with respect to pathogenic potential.

It is well known that cell surface properties, such as hydrophobicity and zeta potential, play an important role in adhesion of micro-organisms to the surface of medical devices (Cai *et al.*, 1994; Anil *et al.*, 2001; Henriques *et al.*, 2002). As a result, we investigated the relationship between adhesion and hydrophobicity and zeta potential parameters (Table 1). The results showed that the water contact angle values obtained for the different *Candida* species were very similar, which differs from the results previously reported by Anil *et al.* (2001), who demonstrated that hydrophobic behaviour varied amongst different *C. albicans* strains, although grown in different conditions. Furthermore, this work showed that *C. parapsilosis*, *C. tropicalis* and *C. glabrata* strains were all hydrophilic and this fact does not affect the adherence abilities. In addition, all the tested strains analysed had similar water contact angle values and this was despite *C. glabrata* exhibiting significantly higher adherence to silicone. Camacho *et al.* (2007) also did not find a correlation between hydrophobicity and adherence for *Candida* species on siliconized latex urinary catheters, demonstrating that cell hydrophobicity alone was not a predictor for the adhesion phenomenon. Since *Candida* cells and silicone (Sousa *et al.*, 2009) are negatively charged, it was expected that adhesion of strains with greater negative zeta potentials would be hindered by electrostatic repulsion. Interestingly, the highest number of adhered cells was found for *C. glabrata* strains, which actually had the higher negative values of zeta potential, thus highlighting that zeta potential is also not primarily responsible for adherence potential. Additionally, according to several authors (Verran & Maryan, 1997; Carlén *et al.*, 2001), micro-organism adhesion is dependent on the surface roughness of the substratum. Depending on the extent of surface roughness, areas suitable for the physical retention of micro-organisms

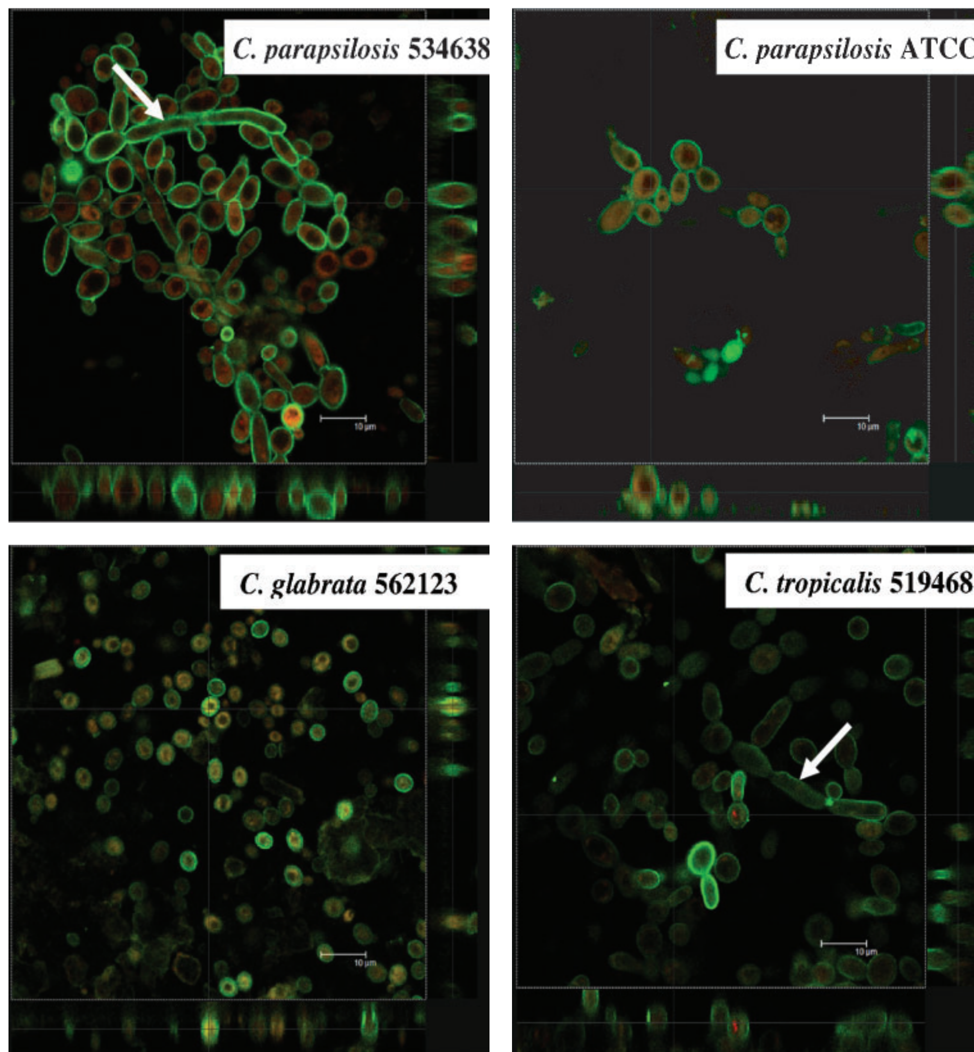

Fig. 3. CLSM images of NCAC species 72 h growth on a silicone surface in the presence of urine. Arrows indicate the presence of hyphal morphologies. Magnification ×600.

to aid colonization are present. Sousa *et al.* (2009) recently demonstrated that silicone exhibits large numbers of depressions and grooves, and since it is known that *C. glabrata* cells are generally smaller (1–4 μm) than *C. tropicalis* (4–8 μm) and *C. parapsilosis* (2.5–4 μm) cells (Odds, 1998) it is tempting to speculate that the silicone rubber coupons used in this study had a surface roughness optimal for *C. glabrata* retention.

Initial attachment of individual cells to a substratum is closely followed by cell division, proliferation and biofilm development (Ramage *et al.*, 2006). Under the conditions used in these experiments and despite extensive adherence of NCAC species, biofilm formation was not subsequently detected (Fig. 2). Nevertheless, it was possible to observe differences among the NCAC species under study regarding total biomass. As found in the adhesion assays, *C. glabrata* colonization yielded a higher total biomass than that of *C. parapsilosis* and *C. tropicalis*. It is known that mature *Candida* biofilms are organized into structured communities embedded within a matrix of extracellular material that is produced by the biofilm cells (Al-Fattani & Douglas, 2006; Silva *et al.*, 2009). Formation of mature biofilms and consequent production of matrix is strongly dependent on environmental conditions, such as medium composition, pH, oxygen, species and strains (Jain *et al.*, 2007; Ramage *et al.*, 2006). In fact, these particular isolates are not capable of forming dense 3D biofilms and producing enough matrix (Fig. 3). However, it should be emphasized that only a few isolates were used and other isolates belonging to the same species may be able to form stronger biofilms. Furthermore, the authors verified that, as previously recognized (Jain *et al.*, 2007; Uppuluri *et al.*, 2009), an increase of sugars (which could be representative of diabetic patients) is a requirement for increased biofilm formation (data not shown). Furthermore, fungal infections

are also more common in patients with diabetes, particularly those involving *Candida* species, occasionally causing dramatic clinical repercussions with obstruction of the urinary catheter (Patterson & Andriole, 1995). However, it is important to stress that the composition of AU was formulated to mimic the natural urine of non-diabetic patients (pH 5.6 with a residual quantity of sugar).

Enumeration of c.f.u. and confocal observation revealed that whilst NCAC species were unable to form dense biofilms on the silicone in the presence of AU (Fig. 3), *Candida* cells remained viable over the 72 h period of study [mean log c.f.u. (cm biofilm)$^{-2}$: 5.3 for *C. parapsilosis*, 5.4 for *C. tropicalis* and 6.1 for *C. glabrata*]. This is highly important as it implies that under appropriate conditions *Candida* can proliferate within the catheter environment and potentially infect vital organs in the host (such as bladder and kidneys).

In summary, this work demonstrates that the tested NCAC species were able to colonize and survive on the silicone surface in the presence of AU. Additionally, it was shown that *C. glabrata* strains exhibited higher colonization of silicone surfaces compared with *C. tropicalis* and *C. parapsilosis*. This finding is potentially significant, not just for UTI in catheterized patients, but also for other patients who have inserted silicone medical devices including those patients who receive transplants or who are admitted into intensive care units. Importantly, such patients would appear to have a higher incidence of colonization with *C. glabrata* than with other NCAC species (Manzano-Gayosso *et al.*, 2008; Guglielmo *et al.*, 1994; Krcmery, 1997).

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