Virulence of *Candida albicans, Trichomonas vaginalis* and *Chlamydia trachomatis* Vaginal Pathogens in Sexually Transmitted Diseases

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Abstract: The present work was designed to study Sexually transmitted infections (STIs) that are spread primarily through person-to-person sexual contact. After isolation and purification of microorganisms from urine, vaginal fluid, urethral discharge, prostate secretions, or urethral scrapings semens, and endocervical smear from 50 controlled groups and 50 patients groups of women and men, they identified as aerobic Gram positive cocci such as *Staph. aureus, Staph. epidermidis, Streptococci spp* and *Sarcinaspp,* Gram negative cocci as *proteus spp* and Gram negative rods as *E. coli*, anaerobic bacteria such as *Bacteroids distasonis, lactobacilli spp*, yeast such as *Candida albicans* were isolated from control women and men groups. However, *Chlamidia* and *Trichomonus vaginalis* were isolated only from patient groups. The results suggest that protease production, germ tube formation and adherence to epithelial cells play an important role in virulence of *Candida albicans*. However, protease production and adherence to epithelial cells were the virulence factors of *Trichomonus vaginalis* and *Chlamidia trachomatis*.

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1. Introduction

Sexually transmitted infections (STIs) are infections that are spread primarily through person-to-person sexual contact. There are more than 30 different sexually transmissible bacteria, viruses and parasites (Slotnick *et al.,* 1963 and Faro., 1991). An extensive and diverse spectrum of pathogenic and non-pathogenic organisms may be observed in vaginal microflora. In spite of the wide-ranging literature on the microbiology of normal and abnormal flora of the vagina, many questions have still not been completely answered (Fleury., 1981and Hammill *et al.,* 1989).Various infectious processes in the vagina are the result of disequilibrium of this flora[1], such as that occurring during pregnancy(McCue *et al.,* 1979 and *et al.,* 1991). The most common STDs infections are caused by *Candida sp., Gardnerella vaginalis, Trichomonas vaginalis* and *Chlamydia trachomatis* (Bauer *et al.,* 1991and . Bosch, 1992)

Most STDs affect both men and women, but in many cases the health problems they cause can be more severe for women (Schneider *et al.,* 1987 and Murta *et al.,* 1997). If a pregnant woman has an STD, it can cause serious health problems for the baby (Moore *et al.,* 1982).Sexually transmitted diseases (STDs), also called venereal diseases, are caused by sexual contact or contact with infected objects such as toilet seats. Sexually transmitted diseases have become a serious health problem (Kinghorn, 1978).Young women are at greater risk than older women for reproductive and health complications caused by STDs (Carne, 1990 and Strand *et al.,* 1993)

Because Sexually transmitted diseases are prevalent in the world, our study advise a sexually active adult to have regular sexual check-ups to identify and diagnose STDs. There are several methods to test for sexually transmitted diseases (or sexually transmitted infections) such as physical examination and laboratory diagnosis when symptoms are visible.

2. Materials and Methods

Specimens

Clinical specimens include urine, vaginal fluid, urethral discharge, prostate secretions, or urethral scrapings semen, and endocervical smear. Vaginal specimens for culture may be either self collected with a swab, or collected by the clinician with a swab or plastic loop for the diagnosis of bacterial, fungal and parasitic infections (Voog *et al.,* 1995).

Diagnosis (Suzanne *et al.,* 2003, Yin *et al.,* 2008 and Martínez 2009:

- Physical Exam to look for symptoms of disease
- Microscopic examination of vaginal secretions
- Culture of male urine or urethral swab

- Polymerase chain reaction (PCR)

Microscopic examination

Identification of the organism on microscopic examination of clinical specimens was done using Wet mount preparation for diagnosis of trichomoniasis in women. Vaginal secretions are obtained from the lateral walls using a swab or plastic loop; in men, any urethral discharge, prostate secretions, or urethral scrapings may be used. The secretions are then suspended in 0.85% normal saline and examined under the microscope.

Culture on Diamond's modified medium was used for diagnosing *T vaginalis* infect In men, microscopy of urine is insensitive, although occasionally organisms are visible in a first-voided morning specimen or a centrifuged specimen. Cultures of urine and urethral swabs are more sensitive.

Polymerase chain reaction (PCR) Anke and Birgit (2008)

Because of the insensitivity of wet mount preparation and the problems with culture methods, nucleic acid amplification methods, such as polymerase chain reaction (PCR), have become increasingly attractive for diagnosing of STIs. PCR has the advantage of requiring only DNA, from either viable or non-viable organisms, and in concentrations as low as one organism per PCR reaction. Microbial samples were treated with enzymes that amplified specific region of the microbe. After amplification, the number of DNA fragments were quantified. PCR were carried out in a total volume of 25 µl consisting of 1x Eurogentec Master Mix, 5 mM $MgCl_2$, Amprease, 115 nM primers and 100 nm probe and 2.5 µl of template DNA. The X^2 test was used for statistical analysis with the significance level set at less than <0.05(Platz-Christensen *et al.,* 1994).

Blood, Urine or Cell Samples

The testing and diagnosis of chlamydia, *Trichomonas vaginalis*, require blood, urine or cell samples to be taken and analyzed.

Chlamydia

Chlamydia can be detected by a urine test or swab sample from the urethra, the cervix, rectum, throat or eye. A visible symptom of chlamydia is inflamed cervix cells or a discharge.

Trichomoniasis

Trichomoniasis is caused by the parasite *Tricohomonas vaginalis*. Trichomoniasis, or trich, can be detected in a physical examination when an unusual discharge is apparent or during a routine cervical smear test. Another method to test for this STD is through a urine sample or a cell sample from the vagina, genital area or urethra. For all of the STDs listed

Hyaluronidase, chondroitin sulphatase and proteinase assay (Baeten *et al.,* 2001).

Hyaluronidase, chondroitin sulphatase and proteinase secretion were tested using Sabouraud glucose agar (Difco, USA). An aqueous solution of 2 mg/ml human umbilical cord sodium hyaluronate (Sigma Chemical Co., USA) was sterilized by Seitz filter (0.02 µm), 4mg/ml bovine trachea chondroitin sulphate type A (Sigma) and 5% of bovine albumin fraction V (Sigma) were sterilized by Millipore filtration (0.02 µm). The substrates were added to cooled media to give final concentrations 400 mg/ml, bovine albumin fraction V was added to give final concentrations of 1%. The final pH was 5.6. Plates were point inoculated and incubated for 3-4 days at 37°C. The plates were flooded with 2 N acetic acid for 10 min. The enzyme activity was visualized by the presence of clear zone around the colonies and determined using the method described by Price *et al.* (1982). Hyaluronidase and chondroitin sulphatase activity (Hy, Ch) was measured in terms of the ratio of the diameter of the colony to the total diameter of the colony plus clear zone around it. When the value of Hy or Ch is below 1 it indicates that the isolate is releasing the enzyme into the medium. Sabouraud glucose agar containing 1% BSA was used for detection of proteinase.

Epithelial cells(Banno *et al.*, 1985)

The urine samples were obtained in the morning from healthy women and centrifuged at 350 rpm for 10 min to harvest the epithelial cells. The sediment was washed twice in phosphate buffered saline (PBS) pH 7.2 and the number of cells were estimated microscopy with a counting chamber, then standardized to 10^5 cell/ml in PBS buffer.

Adhesion assay (Douglas, 1986)

A mixture of equal volumes of epithelial cells (10^5 cell/ml) and *Candida albicans* (10^8 cell/ml) was incubated in plastic tubes on a rotator at 37 °C for 2 hrs. The epithelial-yeast mixture was passed through polycarbonate filters (12 µm pore size) to remove non adhering yeast. Adhesion was evaluated spectrophotometer by measuring the optical density of epithelial cells with adherent yeasts.

3.Results

After isolation, purification and identification of microorganisms from urine, vaginal fluid, urethral discharge, prostate secretions, or urethral scrapings semen, and endocervical smear from 50 control group and 50 patients group of women and men, aerobic Gram positive cocci such as *Staph. aureus*, *Staph. epidermidis*, *Streptococci spp* and *Sarcinaspp*, Gram negative cocci as *proteus spp.* and Gram negative rods as *E. coli*, anaerobic bacteria such as *Bacteroids distasonis* and *lactobacilli spp,*

yeast such as *Candida albicans* were isolated from control and patients women and men groups. However, *Trichomon vaginalis* and *chlamydia* were isolated only from patient groups and as shown in Table 1.

Table 1: Infection by various microorganisms in patients with signs and symptoms in relation to control group

Microorganisms	Women						Men					
	Control		Patients		X^2	*P*	Control		Patients		X^2	*P*
	No	%	No	%			N	%	No	%		
Staph aureus	4	8	7	14	0.9	NS	2	4	2	4	0.0	NS
S. epidermidis	2	4	12	24	8.3	<0.05	3	6	10	20	4.3	<0.05
Streptococci spp	3	6	5	10	0.5	NS	5	10	5	10	0.0	NS
Sarcinaspp	1	2	1	2	0.0	NS	1	2	1	2	0.0	NS
E. coli	-	-	4	8	5	<0.05	-	-	4	8	5	<0.05
Proteus spp	1	2	-	-	1.0	NS		2	-	-	1.0	NS
Bacteroids spp	2	4	8	16	4.0	<0.05	2	4	7	14	4.0	<0.05
Lactobacilli spp	20	40	24	48	0.7	NS	20	40	23	46	0.4	NS
Candida sp	3	6	25	50	0.0	NS	4	8	4	8	0.0	NS
Trichomonas	-	-	12	24	8.3	<0.05	-	-	4	8	5	<0.05
Clamydia	-	-	4	8	5	<0.05	-	-	4	8	5	<0.05

Table 2 shows features of common Infectious vaginitis. Yeast symptoms of itching and burning often overlap with other conditions such as allergic or irritant vulvitis, then the exact signs and symptoms of yeast vulvovaginitis were diagnosed by cultures and microscopic examinations.

Table 2. Typical Features of Common Infectious Vaginitis

	Vulvovaginal candidiasis
Symptoms	
vulvar irritation	++
Dysuria	-
patients –	++
Odor	+ -
Signs	
Labial erythema –Satellite lesions	+
Discharge	+ -
Consistency	Crudy
Color	White
Microscopy	Normal
Epithelial cells	Variable
PMNs/epithelial cell	Gm +ve
Bacteria Gram-	Yeast and Pseudohyphae
Pathogens	
Bimanual examination	
Vaginal tenderness	+
Rugal hypertrophy	+
Adnexal tenderness	-

++ = usually present and may be severe; + = usually present; ± = variably present; – = not usually present and presence should raise concern for other diagnosis; PMNs = polymorphonuclear leukocytes.

Table 3 shows a group of women who had positive cultures for *candida albicans* (some women have positive cultures but no symptoms) signs of vulvar swelling, cracked skin fissures, a reddened

vulva or sores from scratching and a thick, curdy vaginal discharge were infrequent. When the women had their symptoms and findings categorized by whether the culture was positive or negative and whether the microscopic wet-prep was positive or negative, the frequencies in the different groups were interesting.

Table 3: Symptoms and Signs by Test Results

Symptom/ Sign	Culture negative wet-mount negative (presumably not infected)	Culture positive wet-mount negative (uncertain infection)	Culture positive wet-mount positive (presumably has infection)
Symptoms			
Chief complaint of vulvar itching or burning	8%	13%	38%
Vulvar swelling	10%	22%	25%
White discharge	63%	62%	68%
Yellow discharge	23%	26%	22%
Examination findings			
Vulvar swelling/edema	2%	11%	22%
Vulvar redness	21%	32%	72%
Thick curdy discharge (exam)	1%	3%	28%

Morphology of *Candida albicans* strains isolated from vagina of asymptomatic and symptomatic patients:

The results demonstrated non germinative non virulent strains of *Candida albicans* with typical yeast-like form similar to the morphology in their respective in vitro cultures isolated from the vagina of asymptomatic patients (Figure 1).

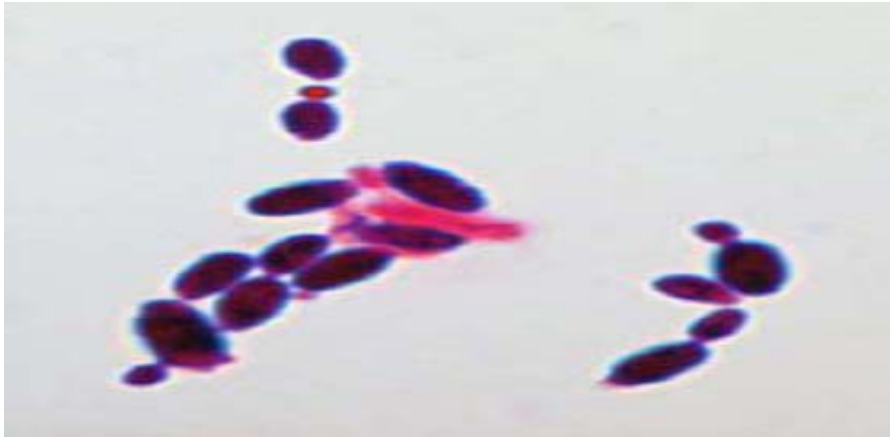

Figure 1: Non virulent strains of *Candida albicans* yeast

The morphology of *Candida albicans* strains isolated from the vagina of symptomatic patients showed long filaments tube of pseudomycelial and germ tube forming cells (Table 4) .

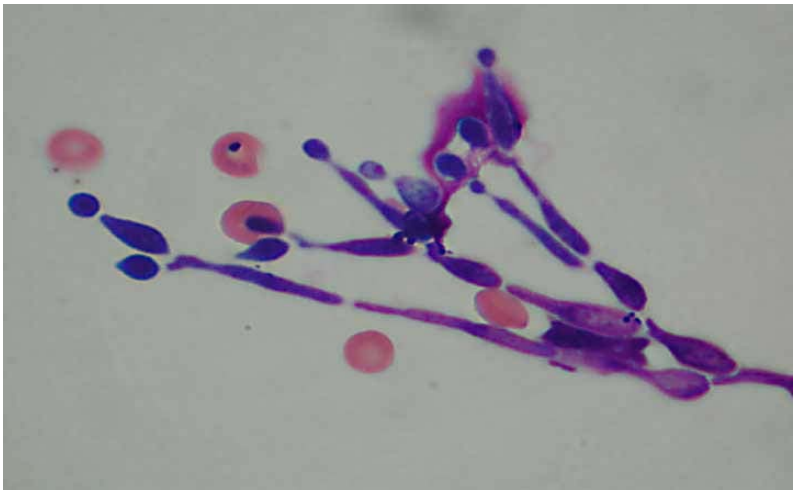

Figure 2: Virulent strains of hyphae and germ tube forming *Candida albicans*

Table 4 morphological characteristics of selected isolates of *C. albicans*

Asymptomatic Strain	Serotype	Morphology			Symptomatic Strain	serotype	Morphology		
		C	H	G			C	H	G
C1					C1				
C2	A	+	-	-	*C2*	A	-	+	+
C3	A	+	-	-	*C3*	A	-	+	+
C4	A	+	-	-	C4	A	-	+	+
C5					*C5*				
C6	A	+	-	-	*C6*	A	-	+	+
C7	A	+	-	-	C7	A	-	+	+
C8	A	+	-	-	*C8*	A	-	+	+
C9					*C9*				
C10	A	+	-	-	C10	A	-	+	+
C11	A	+	-	-	*C11*	A	+	+	+
C12	A	+	-	-	*C12*	A	+	+	+
C13					C13				
C14	A	+	-	-	C14				
C15	A	+	-	-	*C15*	A	-	+	+
C16	A	+	-	-	*C16*	A	-	+	+
C17	A	+	-	-	C17	A	-	+	+
C18					*C18*	A	-	+	+
C19					*C19*				
C20	A	+	-	-	C20	A	-	+	+
C21	B	+	-	-	*C21*	A	-	+	+
C22	A	+	-	-	*C22*	A	-	+	+
C23					C23				
C24	A	+	-	-	*C24*	A	-	+	+
C25	C	+	-	-	*C25*	A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		C	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+

C, *Candida albicans* yeast H, hyphae G, Germ tube.

Hyaluronidase, chondroitin sulphatase and proteinase activity

The comparative study of the enzyme activity revealed that all tested *Candida albicans* isolates from asymptomatic carriers were proteinase, hyaluronidase and chondroitin sulphatase non-producer (Pr^-, Hy^-, Ch^-) except 4 isolates were weak chondroitin producer and 6 isolates were hyaluronidase producer (Table 5). All tested *Candida albicans* isolates from patients were proteinase, hyaluronidase and chondroitin producerexcept *C19* was hyaluronidase non producer (Pr^+, Hy^-, Ch^-) and *C21* was chondroitin sulphatase non-producer (Pr^+, Hy^+, Ch^-) . However, *Trichomonas vaginalis* and *Chlamydia trachomatis* strains were isolated only from patients showed the highest hyaluronidase, chondroitin sulphatase and proteinase enzyme activity (Table 6).

Table 5 Hyaluronidase, chondroitin sulphatase and proteinase production by vaginal *Candida albicans* isolates from asymptomatic carriers.

Isolate	**Hy**	**Ch**	**Pr**	**Isolate**	**Hy**	**Ch**	**Pr**
C1	1.00	1.00	1.00	C14	0.89	0.88	1.00
C2	1.00	1.00	1.00	C15	0.99	1.00	1.00
C3	1.00	1.00	1.00	C16	0.95	1.00	1.00
C4	1.00	1.00	1.00	C17	1.00	1.00	1.00
C5	1.00	1.00	1.00	C18	1.00	1.00	1.00
C6	1.00	1.00	1.00	C19	1.00	1.00	1.00
C7	1.00	1.00	1.00	C20	1.00	1.00	1.00
C8	1.00	1.00	1.00	C21	1.00	1.00	
C9				C22			
	1.00	1.00	1.00		1.00	1.00	1.00
C10	1.00	1.00	1.00	C23	1.00	1.00	1.00
C11	0.98	0.95	1.00	*C24*	1.00	1.00	1.00
C12	0.96	0.91	1.00	*C25*		1.00	
C13	0.93	0.89	1.00		1.00		1.00

Value (relative enzyme activity) lower than 1.0 indicates enzyme activity

Adhesion assay

Figure 3 showed adhesion of three strains of *Candida albicans* (yeast like cells, filamentous cells and germ tube forming cells). Germ tube forming *Candida*

albicans strain revealed the highest percentage of adherence to epithelial cells (highest optical density). In comparison to germ tube forming *Candida albicans* strain, filamentous *Candida albicans* strain showed 60% adhesion. Avirulent yeast like *Candida albicans* strain reported 10 % adhesion. However *Chlamydia* and *Trichomon vaginalis* showed the highest percentage of adherence to epithelial cells (highest optical density) as in Germ tube forming *Candida albicans*.

4. Discussion

Sexually transmitted diseases (STDs) are infections generally acquired by sexual contact. The organisms that cause sexually transmitted diseases may pass from person to person in blood, semen, or vaginal and other bodily fluids (Menday, 2002) . Some of these infections can also be transmitted non sexually, such as from mother to infant during pregnancy or childbirth, or through blood transfusions or shared needles (Donders and Spitz, 2000)

Most STDs affect both men and women, but in many cases the health problems they cause can be more severe for women. If (STI) are not untreated, they can increase the risk of acquiring another STD such as HIV. This happens because an STD can stimulate an immune response in the genital area or cause sores, either of which might make HIV transmission more likely. Some untreated STDs can also lead to infertility and urinary tract infection in men, but often causes no symptoms in men(Schwebke and Morgan, 1997)

Trichomonus vaginalis, *Chlamydia trachomatis* and *Candida albicans* are the most commonly reported organisms cause sexually transmitted diseases. symptoms range from mild irritation to severe inflammation(Schwebke et al, 1999). Thrush, also known as candidiasis, is a yeast infection caused by the Candida species of fungus. Thrush is not technically a sexually transmitted infection, as Candida is a common yeast that is found on the skin and genitals of most people, even those who have not had sex. Candida is usually suppressed by the immune system and the natural bacteria found in the body, but there are many things that can upset the balance and allow Candida to grow. Thrush occurs a

lot less frequently in men (Hill, 1965 and Goldenberg, 2000)

Germ tube and hyphal development of Candida albicans is likely to play a role in pathogenesis of acute Candidal vaginitis. A widely accepted suggestion holds that the yeast form of this fungus colonize the healthy vagina asyptomatically, whereas hyphal or pseudohyphal development is associated with the onset of disease. Mechanism by which hyphal growth can favor fungal infection, such as the increase of fungal adherence to vaginal epithelial cells, have been also postulated(Goldenberg et al., 2002).

Production of the enzyme aspartyl proteinases is also advocated as a characteristic of the most vaginopathic organisms (Bernardis et al., 1990)

Its apparent relevance as a vaginopathic factor for Trichomoniasi, Chlamydia and Candida albicans can be possibly explained by the virulence factors such as secretion of hyaluronidase, chondroitin sulphatase and proteinase enzymes and adhesion ability (King et al., 1980 and Lederman et al., 2008) . Proteinase secretion ofTrichomoniasi, Chlamydia and Candida is likely to play a role in human vaginitis. Hyaluronidase, chondroitin sulphatase and proteinase can affect the permeability of epithelium at the intercellular spaces by attacking the intercellular cementing substances of tissue. As a consequence of this destruction, microbial invasion of the tissue may occur (Rex et al., 2000 and Spinelli, 2010)

Table 6 Hyaluronidase, chondroitin sulphatase and proteinase production byvaginal *Candida albicans,Trichomonasvaginalis*and *Chlamydia trachomatis* isolates from patients

Isolate	**Hy**	**Ch**	**Pr**	**Isolate**	**Hy**	**Ch**	**Pr**
C1	0.52	0.65	0.71	C14	0.93	0.87	0.73
C2	0.55	0.59	0.61	C15	0.59	0.65	0.67
C3	0.61	0.77	0.79	C16	0.87	0.82	0.88
C4	0.76	0.80	0.83	C17	0.57	0.64	0.69
C5	0.68	0.74	0.68	C18	0.89	0.87	0.89
C6	0.88	0.75	0.72	C19	1.00	0.79	0.93
C7	0.72	0.69	0.59	C20	0.90	0.84	0.87
C8	0.79	0.72	0.78	C21	0.75	1.00	0.69
C9	0.64	0.81	0.80	C22	0.87	0.77	0.75
C10	0.69	0.65	0.55	C23	0.66	0.86	0.74
C11	0.84	0.98	0.82	C24	0.95	0.98	0.81
C12	0.84	0.79	0.68	C25	0.85	0.88	0.79
C13	0.69	0.73	00.54				
Trichomonas							
Tr1	0.54	0.55	0.46	Tr12			
Tr2	0.61	0.68	0.52	Tr13	0.62	0.75	0.50
Tr3	0.72	0.72	0.55	Tr14	0.75	0.68	0.62
Tr4	0.55	0.75	0.66	Tr15	0.68	0.58	0.75
Tr5	0.62	0.59	0.55	Tr16	0.58	0.55	0.68
Tr6	0.75	0.78	0.62	Tr17	0.55	0.61	0.58
Tr7	0.68	0.80	0.75	Tr18	0.61	0.72	0.55
Tr8	0.58	0.66	0.68	Tr1	0.72	0.58	0.61
Tr9	0.55	0.55	0.58	Tr20	0.55	0.55	0.57
Tr10	0.61	0.82	0.55		0.45	0.61	0.58
Tr11	0.72	0.55	0.66				
Chlamydia							
Ch1	0.70	0.66	0.52	Ch9	0.55	0.62	0.45
Ch2	0.62	0.55	0.55	Ch10	0.62	0.75	0.60
Ch3	0.66	0.62	0.66	Ch1l	0.75	0.68	0.55
Ch4	0.69	0.75	0.55	Ch12	0.68	0.58	0.68
Ch5	0.71	0.68	0.62	Ch13	0.58	0.55	0.58
Ch6	0.56	0.58	0.75	Ch14	0.56	0.66	0.55
Ch7	0.48	0.55	0.68	Ch15	0.66	0.64	0.61
Ch8	0.64	0.66	0.58	Ch16	0.70	0.58	0.57

Value (relative enzyme activity) lower than 1.0 indicates enzyme activity

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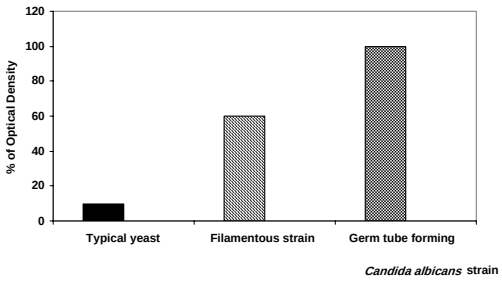

Figure 3: Adhesion of *Candida albicans* to epithelial cells

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