

## Adhesion and biofilm formation of *Candida parapsilosis* isolated from vaginal secretions to copper intrauterine devices

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### ABSTRACT

**Introduction:** *Candida parapsilosis* is one of the main species that is able to adhere to forming biofilms on inert materials. Adhesion is the first step towards the colonization and invasion of host cells during the infectious process. Among the infections, vulvovaginal candidiasis is increasingly common. The objective was to evaluate the profile of adherence and biofilm formation of eight isolates of *C. parapsilosis* on the metal used in intrauterine devices (IUDs). **Methods:** Eight strains of *C. parapsilosis* presenting strong adhesion and biofilm formation properties were isolated from vaginal secretions in a previous study. To assay the adhesion and biofilm formation, copper fragments were made and cultivated in tubes containing 3 mL of phosphate-buffered saline and incubated for 6 and 24 h at 37 °C to evaluate biofilm formation. After incubation, the intensity of adherence and of biofilm formation on copper fragments were determined by performing a colony count. **Results:** All isolates were able to form biofilms and the isolate Cp62 showed many cells joined in a planktonic mode forming biofilms. The use of an IUD is one of the main factors that favors vulvovaginal candidiasis, and the presence of copper in this device increases the chance of recurrent vulvovaginal candidiasis (CVVR) due to the ease with which species of the genus *Candida* can adhere to inert surfaces. **Conclusion:** This research showed that the clinical isolates studied adhered to IUD copper fragments and formed biofilms, further increasing their virulence.

**KEYWORDS:** *Candida parapsilosis*. Adhesion property. Formation of biofilm.

### INTRODUCTION

The normal vaginal microbiota is predominantly comprised of lactobacilli (bacilli Döderlein hosting), which promote the proper acidity of the vaginal environment (~ pH 4.5); this hinders the proliferation of most microorganisms that can eventually be pathogenic. Despite this balance, several factors contribute to the colonization of vagina by *Candida* species, sometimes favoring the onset of symptoms or the occurrence of asymptomatic infections<sup>1,2</sup>.

It is estimated that vulvovaginal candidiasis (VVC) is the second most frequent cause of vaginal symptoms, representing 17 to 39% of the cases, and bacterial vaginosis (BV) corresponding to about 50%<sup>2</sup>. Around 75% of women present with vaginal discomfort at any stage of life, and among these cases, 50% have other episodes of VVC and 5% have recurrent vulvovaginal candidiasis (CVVR). This medical condition is caused by a single agent, but the mechanism going

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from transformation to colonization and to infection is multifactorial<sup>3</sup>.

*Candida parapsilosis* is the second most common species isolated in different types of candidiasis<sup>4-6</sup>. The frequency is 15.5% in North America, 23.4% in Europe, and 16.3% in Latin America. In the United States, VVC is caused by the *Candida* species *albicans* and is not related to the widespread use of oral antifungals. In a study of 211 women in India with suspected VVC, 50 cases were confirmed by culture of vaginal secretions (23.7%). The identified species were *C. glabrata* (45.1%), followed by *C. tropicalis* (23.5%), *C. albicans* (17.6%), *C. krusei* (9.8%) and *C. parapsilosis* (3.9%). In addition, there was no association between *C. glabrata* and *C. albicans* in 2% and 0.5% of the positive cultures from the cases of VVC<sup>7</sup>.

In general, *C. albicans* is responsible for 85 to 90% of cases, followed by *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis*; moreover, *C. albicans* is associated with coinfections and recurrent or chronic cases of the disease. In women with VVC, clinical manifestations vary from asymptomatic colonization to a severe, symptomatic acute infection<sup>8,9</sup>.

Recurring causes are related to the symptomatic stage along with the vaginal microbiota and the predisposing factors that define the host susceptibility through interference with the population of lactobacilli, such as the use of antibiotics, reducing the population of *Lactobacillus acidophilus*; the use of spermicide; the use of oral contraceptives and antifungals without prescription medications<sup>10,11</sup>, and other factors such as an increase in temperature, changes in the humidity, and local mucosal trauma, caused by the use of excessively tight clothing and underwear, especially the ones made with synthetic fibers. All of these factors can lead to the development of candidiasis<sup>3</sup>.

A factor that can facilitate the colonization of *Candida* is the use of intrauterine devices (IUDs), which can intensify the woman's susceptibility for vaginosis due to virulence factors of the *Candida* species<sup>12-15</sup>. Several studies have shown that *Candida* species can easily adhere to and form biofilms on inert surfaces, such as glass, metals, silicon, plastics, and acrylics<sup>16,17</sup>.

The ability of accession is the first step in developing VVC. In this mechanism, proteins called adhesins mediate the recognition and binding of fungal cells to cells and to inert surfaces<sup>18</sup>. Additionally, formation of a biofilm is intrinsically favored by the accession process, as well as by the association with microbial cells, which is characterized by a specific gene expression pattern<sup>19-21</sup>. This process occurs at different stages including the initial adhesion and colonization that is followed by cell division, proliferation,

and the biofilm maturation, which can include yeast forms and filaments that are suitable for the colonization of inert surfaces.

Thus, the objective of this study was to evaluate the intensity of the adherence and the biofilm formation of *C. parapsilosis* on copper that is present in IUDs.

## METHODS

The study was descriptive with experimental and prospective characteristics that covered quantitative analysis of data. For the experiments, eight isolates of *C. parapsilosis* (vaginal secretion) were used. They were kindly provided by the laboratory of endemic and parasitic diseases of Ceuma University.

From the isolates, a standard solution was prepared from the 24-h colonies that were grown in Sabouraud dextrose medium (SDA) with chloramphenicol (Acumedia). After this period, they were resuspended in a 0.85% saline solution and standardized to approximately 10<sup>6</sup> CFU/mL, in accordance with a turbidity of 0.5 in a McFarland range tube (CLSI; Clinical and Laboratory Standards Institute, NCCLS, created in 2007)<sup>22</sup>.

To assay the adhesion and biofilms, copper fragments measuring 0.5 mm in diameter (made from model TCu 380A IUDs, adopted by the Ministry of Health for patients, based on SUS) were washed with sterile distilled water, and added to a tube containing 3 mL of PBS sterilized at 121 °C in 1 x 15 min intervals.

Subsequently, 100 µL of the fungal suspension was added to each tube containing the fragments for the two tests. They were incubated for 6 and 24 h to evaluate biofilm formation at 37 °C.

After the incubation period, each fragment was washed 3 times with sterile saline, carefully placed in tubes containing 3 mL of 0.85% saline solution and vortexed for 30 s. From these samples, 100 µL aliquots were added in duplicate to plates containing SDA middle, and the inoculants were spread using a Drigalski spatula. After 24 h of incubation, the number of colony forming units (CFUs) were determined for 0.1 mL of *C. parapsilosis* recovered from each fragment.

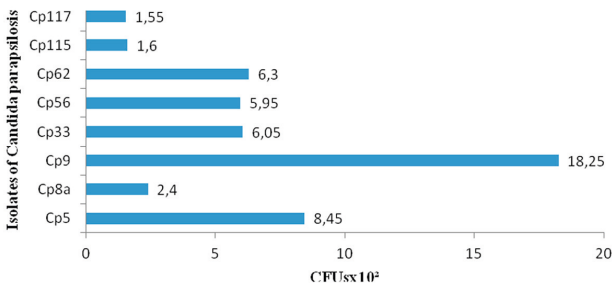
Adhesion and biofilm formation were classified by the material, according to the cell adhesion, which was considered to be negative for samples that did not show CFUs at the end of the process. Weak adhesion samples showed growth between 1 and 199 CFUs; moderate samples had CFUs between 200 and 499; strongly adhering samples had CFUs between 500 and 1,000 CFUs; and very strong adhesion samples had CFUs of more than 1,000 CFUs.

Results were analyzed through the transcription of collected data to graphs and tables.

**RESULTS**

**Adhesion test**

The eight isolates of *C. parapsilosis* used in this study were able to adhere to copper, as shown in chart 1, and a discrete difference in the number of attached cells was observed. Of note, isolate Cp9 adhered more to the copper fragment compared to other isolates, with CFUs of  $18.25 \times 10^2$  (Figure 1).



**Figure 1** - Distribution of the number of CFUs isolates adhered to copper

Regarding the grip strength (Table 1), we noted that seven of the isolates (Cp5, Cp8A, Cp33, Cp56, Cp62, Cp115, and Cp117) had weak intensities, and one isolate (Cp9) had a moderate intensity.

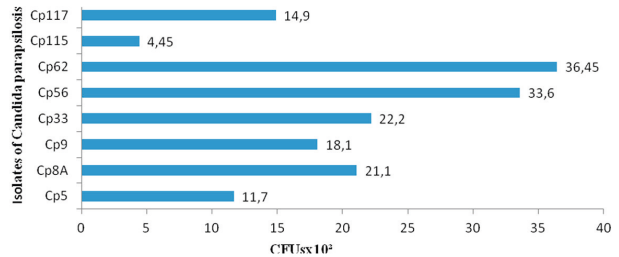
**Table 1** - Distribution of the adherence intensity of *Candida parapsilosis* isolates to copper

Isolates	Intensity adherence
Cp5	Weak
Cp8A	Weak
Cp9	Mild
Cp33	Weak
Cp56	Weak
Cp62	Weak
Cp115	Weak
Cp117	Weak

**Biofilm assay**

Our results showed that the eight isolates of *C. parapsilosis* formed biofilms on copper. Among these, isolate Cp62 showed the highest number of cells in the biofilm ( $36.45 \times 10^2$  CFUs), followed by isolates Cp56 ( $33.6 \times 10^2$  CFUs) and Cp33 ( $22.2 \times 10^2$  CFUs). For the

other isolates, we observed 1.7, 21.1, 18.1, 4.45, and  $14.9 \times 10^2$  CFUs for Cp5, Cp8A, Cp9, Cp115, and Cp117, respectively, for formation of a biofilm on copper (Figure 2).



**Figure 2** - Distribution of the number of cells per CFUs x 10<sup>2</sup> in the formation of biofilm isolates of *C. parapsilosis*

Regarding the intensity of biofilm, after calculating the estimated number of cells that remained attached for 24 h, which are suggestive of biofilm, the number of cells indicated a weak profile biofilms (Table 2).

**Table 2** - Distribution of the number of cells and biofilm intensity to copper

Isolates	CFu	Intensity to copper
Cp5	11,7	Weak
Cp8A	21,1	Weak
Cp9	18,1	Weak
Cp33	22,2	Weak
Cp56	33,6	Weak
Cp62	36,45	Weak
Cp115	4,45	Weak
Cp117	14,9	Weak

**DISCUSSION**

The ability to adhere to surfaces with different intensities can be related to the fact that *C. parapsilosis* consists of three different, closely related complexes. The genetic characteristics allow their separation and classification as *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*<sup>23</sup>.

Studies claim that *C. parapsilosis* has high adhesion to biomaterials due to the ease with which it adheres to various types of surfaces such as plastic, glass, central venous catheters, urinary catheters and other dispositives used in situations where there is a need for prolonged or permanent access. Additionally, as the use of IUDs increases, the chance of colonization by these isolates increases, as does the persistence of CVV<sup>24</sup>.

As already known, a biofilm is a complex layer of cells that is associated with areas that together form an array, giving the organism protection from the host's defenses, as

well as antifungal activity and several species of *Candida*, mainly *C. parapsilosis*, forming biofilms on different inert surfaces, like the IUD<sup>11</sup>. In our study, the biofilm had a weak intensity after 24 h; however, an IUD remains inserted in the intrauterine cavity for a long period of time and this result suggests that it can contribute to VVC recurrence.

In a similar study using central venous catheters, Titton *et al.*<sup>17</sup> analyzed the formation of biofilms by *C. albicans*, *C. tropicalis*, and *C. parapsilosis* and compared the results between species, and they found that *C. parapsilosis* showed the highest rate of colonization, compared to *C. albicans* and *C. tropicalis*, after 24 and 48 h of incubation.

Corroborating our work, Segal *et al.*<sup>12</sup> and Auler *et al.*<sup>14</sup> studied the formation of biofilms in IUD and reported that women who use IUDs are susceptible to episodes of CVV, especially at the insertion site because all devices studied presented with *Candida* spp. biofilms.

## CONCLUSION

Results of this research show that the clinical isolates studied are able to adhere to the copper of IUDs and form biofilms, further increasing their virulence. In this way, these results imply a high pathogenicity for the virulence factors of this species, and it may have a significant role in the use of copper IUD.

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