

Adherence of *Pseudomonas aeruginosa* and *Candida albicans* to urinary catheters

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Summary In this study, the in vitro adherence capabilities of *Pseudomonas aeruginosa* and *Candida albicans* clinical isolates to urinary catheters were investigated. Quantitative analysis was performed by colony-forming unit counts and scanning electron microscopy. Results demonstrated that the adherence of *P. aeruginosa* to urinary catheters was enhanced in the presence of *C. albicans*, while *C. albicans* adherence was not significantly affected. Further investigations are warranted to fully understand the pathogenic potential of their interaction in order to aid in the design of novel strategies for the prevention and treatment of catheter-related UTIs.

Key words Biofilm, *Candida albicans*, Interaction, *Pseudomonas aeruginosa*, Urinary catheter, Urinary tract infections

Adhesión de *Pseudomonas aeruginosa* y *Candida albicans* a catéteres urinarios

Resumen Se investigó la interacción entre aislamientos clínicos de *Pseudomonas aeruginosa* y *Candida albicans*, y su capacidad de adhesión in vitro en catéteres urinarios. Fue realizado análisis cuantitativo a través del cómputo de unidades formadoras de colonias y microscopía electrónica de barrido. Los resultados mostraron que el desarrollo de *P. aeruginosa* sobre catéteres urinarios fue mayor con la presencia de *C. albicans*, mientras que la presencia de la levadura no fue afectada significativamente. Son necesarios más estudios para comprender mejor la patogénesis de estos microorganismos y para establecer estrategias para el control y el tratamiento de las infecciones urinarias vinculadas al uso de catéteres.

Palabras clave Biopelícula, *Candida albicans*, Interacción, *Pseudomonas aeruginosa*, Catéter Urinario, Infecciones Urinarias

Hospital-acquired infections continue to be a serious health problem. Prolonged hospitalization, broad-spectrum antimicrobial therapy, management of patients and invasive procedures are all factors that contribute to their occurrence [1].

Among nosocomial infections, urinary tract infections (UTIs) are the most common and are usually associa-

ted with urethral catheterization [12]. Microbial contamination of the catheter rapidly leads to the formation of biofilm [7], and it plays an important role in the pathogenesis, treatment and prevention of urinary tract infections [10].

Pseudomonas aeruginosa and *Candida albicans* are two of the most important agents of nosocomial infections and are frequently isolated together in catheter-associated urinary tract infections (CAUTIs) [8]. Hogan & Kolter [5] observed that in mixed cultures of these two microorganisms, the bacteria form a dense biofilm on the filamentous form of the fungus. This ability to co-adhere may explain the prevalence of these two species in CAUTIs. To that end, the objective of this study was to evaluate the effect of the interaction between *P. aeruginosa* and *C. albicans* on their ability to adhere to urinary catheters in vitro.

One isolate each of *P. aeruginosa* and *C. albicans*, obtained from urine of a patient hospitalised at Maringá Regional University Hospital, was identified by the MicroScan - AutoScan-4 (Dade Behring, USA) automated system and maintained in a freezer at -20 °C in Tryptic Soy Broth (TSB, Difco, USA) with 10% glycerol, until the time of their use.

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Prior to experiments, *P. aeruginosa* and *C. albicans* were revived in culture and their purity confirmed. A bacterial suspension containing approximately 10^7 colony forming units/ml in sterile physiological solution (SPS) was adjusted by spectrophotometer (Baush & Lomb). Similarly, a yeast cell suspension was prepared from cultures grown in HFM7 medium [6], and adjusted to a density of 10^5 colony forming units/ml [9].

Standardized suspensions of each microorganism were diluted in freshly collected non-sterilized human urine from a healthy donor, which served as a conditioning medium. Sections of siliconized latex Foley urinary catheters (Rusch Gold, Malaysia) measuring 1.5 cm were placed in contact with either pure or mixed suspensions of these microorganisms and incubated for 1 h at 37 °C with continuous agitation. After washing, the strongly adhered microorganisms were removed by agitation with glass beads. The resulting suspension was diluted and an aliquot was seeded, in triplicate, in plates containing CLED Agar and Sabouraud Dextrose Agar (SDA). Each assay was performed in triplicate and repeated at three different times. Similar assays were performed using SPS as a conditioning medium and incubated for 15 h.

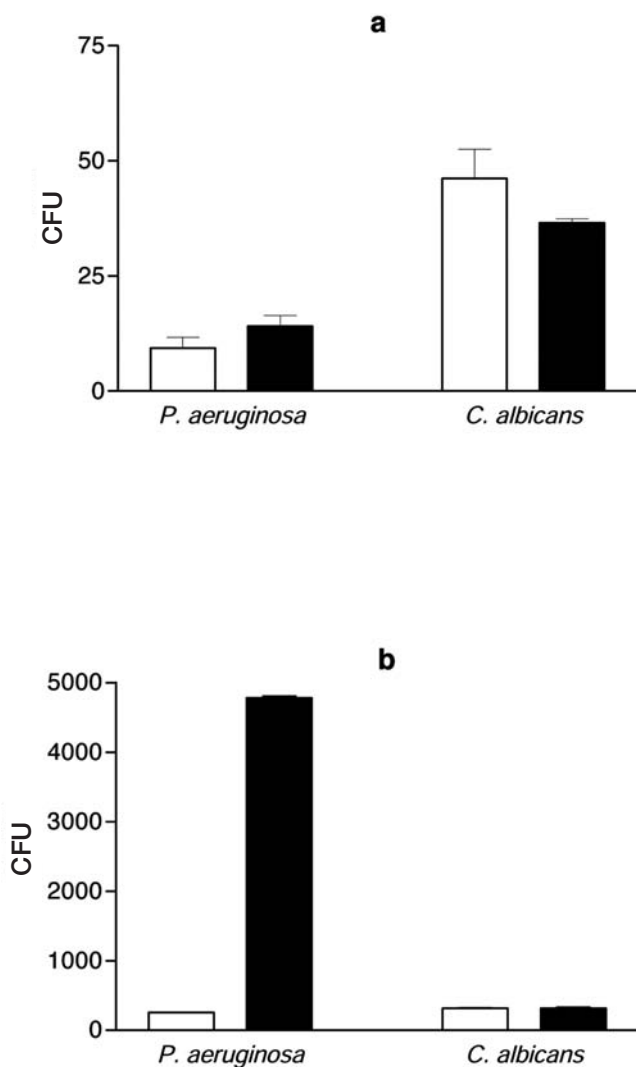


Figure 1. Colony-forming unit (CFU) counts, obtained from adherence assays on urinary catheters, for *P. aeruginosa* and *C. albicans* separately (□) and in association (■), following 1 h (a) and 15 h (b) of incubation.

Scanning electron microscopy (SEM) from co-culture of both microorganisms in HMF7 or Sabouraud Dextrose Broth (SDB, Difco, USA), incubated for 48 h at 37 °C and also on catheter sections incubated for 20 days at 37 °C was visualized in a Shimadzu Superscan SS-550 after adequate preparation [3].

The results were statistically analyzed by “Graph Prism” program version 3.2, using the Student t test with values of $p < 0.05$ considered to be statistically significant.

Results of colony-forming unit counts (CFU) from adherence assays of the two microorganisms, both separately and in association, on the sections of the urinary catheters are demonstrated in figure 1. Bars represent the means of all assays performed. No significant differences between pure or mixed cultures on adherence of both species to the catheter were observed after 1 h of co-infection ($p = 0.09$) (Figure 1a).

In contrast, following 15 h of co-infection with *C. albicans*, a significant increase in the number of adhered cells was observed for *P. aeruginosa* ($p = 0.0001$) whereas no differences in adherence of *C. albicans* was observed ($p = 0.15$).

The interaction between these microorganisms with the adherence of the bacteria to the yeast was also shown by the SEM analysis of the co-cultivation (Figure 2) and by in vitro assays of adherence to the surface of the urinary catheters (Figure 3).

Yeast filamentation was stimulated in HFM7 medium prior to performing the adherence assays in order to provide ideal conditions for the adherence of the bacterium to the yeast. SEM analysis of the interaction in both liquid medium (Figure 2) and on catheter surface (Figure 3), demonstrated that *P. aeruginosa* adhered extensively to *C. albicans* yeast, as well as hyphae forms. These findings

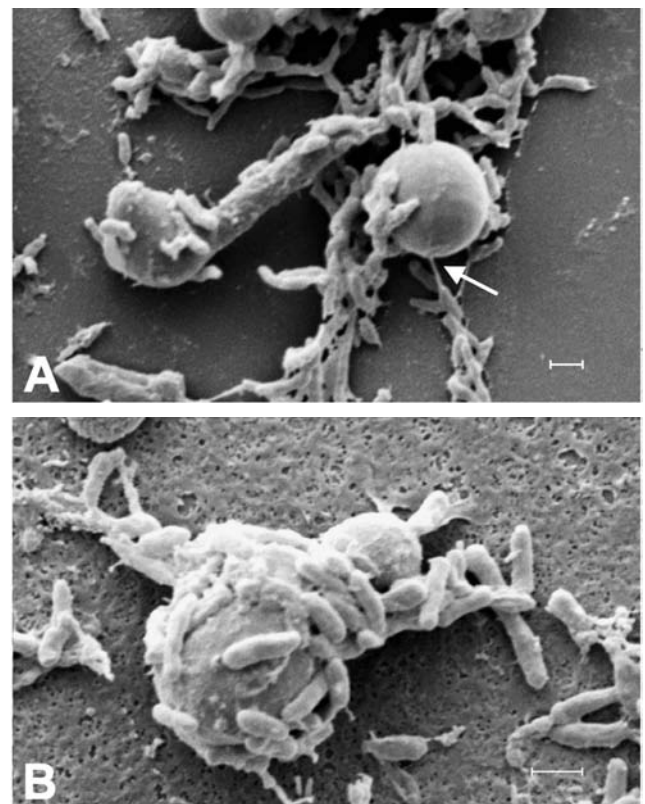


Figure 2. SEM images showing the interaction between *P. aeruginosa* and *C. albicans* following 48 h incubation in HFM7 medium (A) and SDB (B). Bar = 1 μm.

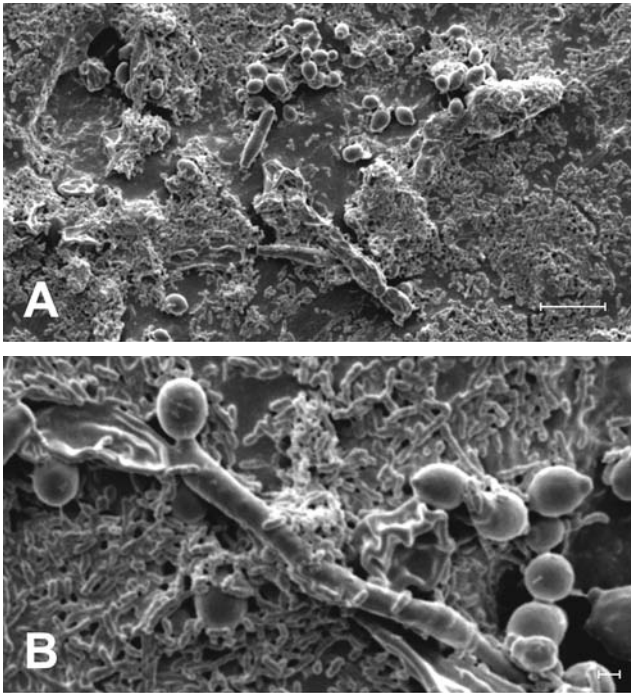


Figure 3. SEM image of *P. aeruginosa* and *C. albicans* biofilm formed on urinary catheters following 20 days of incubation. (A) A dense biofilm containing a large quantity of *P. aeruginosa* cells in interaction with both filaments and blastoconidia of *C. albicans*. Bar = 10 μ m. (B) Interaction of the bacteria with the filament of the yeast. Bar = 1 μ m.

are in accord with El-Azizi et al. [4] who showed interaction between different bacteria, including *P. aeruginosa*, and *C. albicans* and the formation of mixed biofilms on vascular catheters.

The frequent simultaneous isolation of *C. albicans* with *P. aeruginosa* from cases of CAUTIs was the basis for the selection of these two organisms in this present investigation as it may indicate an in vivo interaction. Bacteria are frequently found together with species of *Candida* in natural polymicrobial biofilms and it is probable that interspecies interactions occur among the adherent cell populations [2]. Furthermore, biofilms formed on the surface of urinary catheters are significant contributing factors to CAUTI [11].

In conclusion, this study demonstrates the existence of an interaction between *P. aeruginosa* and *C. albicans* where *P. aeruginosa* was shown to adhere to *C. albicans* in vitro. The interaction between these two microorganisms in vivo could increase the risk and severity of urinary infections contributing to the difficulty of their eradication. In addition, this association could lead to complications and the dissemination of these microorganisms prolonging hospitalization time and increasing costs.

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